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Influence of Diet and Genetic Strain on Desirable Flavors in Farm-Raised Catfish.

Carol Ann Kelly

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**INFLUENCE OF DIET AND GENETIC STRAIN ON
DESIRABLE FLAVORS IN FARM-RAISED CATFISH**

A Dissertation

**Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy**

in

The Department of Food Science

by

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ABSTRACT

This farm-raised catfish quality study measured desirable flavor attributes, *Chickeny, Nutty, Fat Complex, Corn, Sweet*, with the objective to determine effects of feed ingredients, time-on-diet and genetic strain. Overall flavor impact differences were tested by triangle tests. The diets were Casein-base reference feed by itself or partially substituted with: 10% catfish meal, 10% meat and bone meal, 10% rice bran, or 3% menhaden oil and commercial feed formulation. Fish were grown indoors to minimize environmental flavors for 70, 160, 210 and 318 days. The genetic strains evaluated were channel, albino channel, blue, hybrid channel x blue, and black bullhead catfish. These fish were stocked and fed commercial fingerling feed for no less than 14 days. Blended individual fish samples were prepared for trained descriptive (n=9) and triangle test panels (n=18).

The descriptive analyses showed no significant differences due to feed, time-on-diet, or genetic strain. Differences found were not greater than the minimum detectable differences set by a preliminary power analysis. Triangle tests revealed black bullheads to be different from all other genetic strains, as was expected. Commercial reference catfish were found to be different possibly because of a slight off-flavor that was a cue to panelists. Inconsistent overall impact in descriptive and triangle evaluations indicates small differences exist that would likely be at the same intensity or masked by common flavors from pond influences. Fillet fat content was consistent with other reports, and the lack of flavor differences with increasing

time-on-diet suggested that longer growing time to develop flavor is not warranted.

This study supports producer practices of least-cost formulation.

Odor analysis by gas chromatography-olfactometry was performed on selected treatments to explore patterns of impact odorants from catfish oil extracts. An intensity method was performed by four panelists. Twenty compounds were found that met the criteria that at least one panelist rated a *moderate* intensity or higher. All compounds have been found in animal and vegetative products. The three most consistent stimuli perceived were *green grassy*, *mushroom* and *mothballs*. Canonical correlation of the reliability of odor compound data to predict flavor-by-mouth characteristics did not find any significant relationships.

CHAPTER 1 INTRODUCTION

A common reaction when an individual is asked about their preference on a menu for catfish is an emphatic opinion. The response may be a determined “like” or “dislike,” and might express a clear reason why the person would not consider even trying catfish. “It’s trash fish...” or “...aren’t they bottom-feeders?” may be the response. The topic of a muddy-type taste may come up. Some like it; some don’t. Actually, the person’s beliefs may be from information they had heard or read, not from personal experience. In my endeavors, each individual I asked had a definite comment about their view of catfish. The comment usually covers the whole topic of catfish, rather than distinguishing between farm-raised and wild catfish. But, those people asked had a context about catfish as well as a formed opinion.

These opinions are not representative of the facts gathered by sensory experience. High quality catfish flavor and texture are not fishy or boney, and they are well-suited as an ingredient in many recipes. Both freshwater and saltwater catfish species are common worldwide. The consumer may find that several qualities of edibility change with each animal. Season and location of capture are influences on catfish flavor quality known to steady customers. It has not been considered a problem, though, because people considered it typical of catfish. If it tasted slightly vegetative or muddy, in addition to its regular flavors, that was generally accepted.

Flavor variability due to external and internal factors also occurs in other muscle-food and livestock products. Livestock producers, like catfish farmers, desire

to manage as many final product characteristics as possible. For catfish, the presence and balance of certain flavors is important in attracting new and former customers. This expectation of desirable flavor balance is crucial in maintaining and increasing catfish sales growth. Success in achieving this expectation has resulted in farming of channel catfish, *Ictalurus punctatus*, as the leading aquaculture industry in the United States.

As geographic markets for channel catfish and the industry grew, the interpretation of catfish flavor-impact underwent a consequent change. Most consumers in the early stages of the growth were in the southern United States, and most products were captured locally or within that region. New end-users gave feedback that some flavors were unpredictable. They out-balanced the mixture of low intensity, chickeny flavors and muddy/earthy, musty tasting. They were considered undesirable, and the phrase, “off-flavors” in catfish, was coined.

Farm-raised catfish flavor issues have focused on the off-flavors because of their economic impact. The primary metabolites causing perceptions of muddy/musty flavor have been identified as geosmin and 2-methylisoborneol (MIB). The compounds are derived from indigenous bacteria and algae in pond environments. Geosmin and MIB are toxicologically safe at common levels, but the episodes through which they occur are highly unpredictable. The problem related to catfish sales is that muddy/musty flavors are uncharacteristic, unpleasant, linger in aftertaste, and resist masking by recipe ingredients. Flavor-checking procedures at processing plants (Johnsen, 1995) have been established to manage the presence of off-flavors in

commercial catfish products. While the problems have been minimized, they have not been fully eliminated.

Much less research has focused on the innate, underlying flavor constituents that form the matrix against which off-flavors are perceived. An early survey of catfish characteristics listed flavors and textures related to pond and storage conditions (Maligalig et al., 1973). The majority of sensory evaluations have been based on preference scales in small groups, with detailed catfish sensory data published in later stages (Johnsen and Kelly, 1990; Chambers and Robel, 1993).

Farm-raised catfish is a value for the price. It has a role as an economical, low-fat, protein source. Associated with the fat portion of muscle foods, many desirable flavor traits are often carried in incorporated fat or marbling (Lindsay, 1985). Flavor impact has been attributed to characteristic compounds from muscle lipids in meats and fish (Karahadian and Lindsay, 1989). Catfish fillets are composed of an average 6-8% fat (Nettleton, 1990). A portion of this study aimed to determine whether the same principle applied in farm-raised catfish. That is, if overall fillet fat quantity would be translated into higher perceptions of desirable flavors.

Characteristic flavor of catfish was noted as having influence on its marketability (Johnsen, 1989). The influence of feed on flavor is one major factor among farm practices. Aquaculture businesses have considered the practicality of least-cost feed formulations to enhance profitability and quality. Least-cost formulation utilizes seasonally fluctuating, low-price nutrient sources in fish feed, if the mixture meets the fundamental growth needs of the fish. To test the practice of

least-cost formulation, most sensory studies have reported the effect of underutilized materials on catfish quality. More common, highly available nutrients, *e.g.* soy products, would have the most competitive advantage. Few descriptive sensory studies of catfish flavor have been conducted (Chambers and Robel, 1993).

Johnsen and Dupree (1991) reported the flavor impact of 20 common feed ingredients. The catfish were grown on semi-purified casein-base or casein plus ingredient-substituted feed. The catfish were grown for 60 days to a final weight of 150g (one-third pound). The five attributes analyzed, *Chickeny, Nutty, Fat Complex, Corn, Sweet*, were proposed as the primary desirable flavors in catfish, and few differences were found due to feed ingredient. An observation made beyond the objective of that study led to the research reported here. The Reference-Casein fish used in the 10-month catfish storage study had been on diet for 300 days. These catfish had significantly higher intensities of the five desirable flavors analyzed than any of the treatments during the storage periods (Johnsen and Dupree, 1991). It was speculated that time-on-diet may be a factor. Further testing of four prior ingredients that had exhibited the largest flavor intensity differences and the Reference-Casein base diet as the structure of this investigation.

A collaborative network of facilities was available to include examination of desirable flavors in several catfish genetic strains. These experiments were planned on the null hypothesis that catfish desirable flavor intensities are not affected by feed ingredient or genetic strain. Alternatively, the evaluations were designed to determine if there was an effect on desirable flavor intensities by feed or genetics. The primary

flavor impact evaluations were performed by trained sensory descriptive panel and gas chromatography-odor analyses.

CHAPTER 2 REVIEW OF LITERATURE

Background Catfish Production Studies

The farm-raised catfish industry harvested over 215, 000 metric tons of edible products valued at \$365 million dollars in 1996 (Anonymous, 1996). The industry is comprised of a network of interrelated agricultural support activities that was stimulated by husbandry advances at fish hatcheries (Dupree, 1966; Redmayne, 1989). Technological understanding of aquaculture methods through practical experience and research helped the industry advance. In United States aquaculture, channel catfish (*Ictalurus punctatus*) exceeds all other species in quantity produced (Redmayne, 1989).

The introduction of seine-net practices to harvest ponds successively without draining and use of pelletized catfish feed were major advances to produce reliable harvests and higher yields (Johnsen, 1989; Stickney, 1994). Additional knowledge such as the contribution of catfish products to human nutrition (Nettleton *et al.*, 1990), typical storage and processing requirements (Silva, 1991), and building of trade supports helped the industry grow to its current place in United States aquaculture.

Robinson (1989) has summarized research efforts in fundamental areas of farming channel catfish in the early growth phase of the industry. Husbandry research has focused on catfish growth influenced by various nutrients (Dupree, 1966; Dupree and Halver, 1970; Stickney and Andrews, 1971, 1972; Maligalig *et al.*, 1973, 1975a, 1975b; Page and Andrews, 1973; Smith and Lovell, 1973; Garling and Wilson, 1976;

Dorsa *et al.*, 1982; Gatlin and Stickney, 1982; Dupree *et al.*, 1979; Wilson and Poe, 1985; Bai and Gatlin, 1993; Robinson and Li, 1997). Similarly, yield and growth (*i.e.*, dress-out percentage) have been key focal points of several studies (Manthey *et al.*, 1988a; Tidwell, 1987; Silva *et al.*, 1993; Webster *et al.*, 1993; Conrad *et al.*, 1994; Robinson and Li, 1997). Investigations of product quality issues include determination of fillet nutrient composition due to processing methods (Boggess *et al.*, 1971), ω -fatty acid content (Lovell, 1988), and proximate composition differences comparing farm-raised to wild catfish (Chanmugam *et al.*, 1986; Nettleton *et al.*, 1990; Nettleton, 1990).

Johnsen (1989) reviewed pre-storage catfish flavor quality influenced by genetics, diet and environmental conditions. His report described the status of the industry and the scope of the off-flavor problem. Many aspects of post-processing stability related to chilled storage have been studied (Boggess *et al.*, 1971; Gibson and Worthington, 1977; Tidwell, 1987; Manthey *et al.* 1988b; Przybylski *et al.* 1989; Huang *et al.*, 1991; Huang *et al.*, 1992; Silva *et al.* 1993; Freeman and Hearnberger, 1994; Huang *et al.*, 1994; Silva *et al.*, 1994; Kim *et al.*, 1995; Brannan and Erickson, 1996). Reddy *et al.* (1997) and Kim *et al.* (1995) reported catfish fillet microbiological quality issues and their relation to shelf-life.

Early Sensory Methodology using Fishery Products

Parallel to catfish industry growth was the use of quantitative sensory techniques for all finfish. Flavor analyses by semi-trained panels were published for marine fish along with compositional data (Kapsalis, 1980; Prell and Sawyer, 1988).

These findings represented years of sensory method development for fishery industries. Flavor characteristics have been established as important quality factors in determining consumer acceptance of commercial fish products (Wesson *et al.*, 1979; Sawyer *et al.*, 1988; Robinson, 1989).

Finfish edibility characteristics using a closed-end scale (Jahncke *et al.*, 1988), the influence of corn (Wu *et al.*, 1996), or fish oil feed ingredients (Morris *et al.*, 1995) have also been studied. Particular studies objectively addressed issues such as within fillet sampling variability. A semi-trained panel compared precise sections of rainbow trout fillets (Smith *et al.*, 1988). The sampling protocol was conducted quantitatively, but the sensory scoring method utilized preference scales that have less discriminatory power between treatments. Most recently, terminology to describe many freshwater species was published by a trained multi-product panel (Chambers and Robel, 1993).

In the 1970s and 1980s, the catfish industry became more established and began to market their products beyond the original geographic areas. To determine flavor, texture and overall acceptability characteristics of farm-raised catfish, sensory evaluation was sometimes added as a tool in research designs. Various procedures from acceptance/preference scales to complexed-term rating, *e.g.* “overall flavor intensity”, have been used to evaluate catfish sensory attributes.

The Concept of Desirable Flavors in Farm-Raised Catfish

There has not been a comprehensive, industry effort to understand a range of acceptable flavor characteristics. Many of the studies reported at the annual Catfish Processors’ Workshop (Silva, 1991) have also included hedonic rating of farm-raised

catfish. The participants were from the geographic region where consumers are very accustomed to the product. Therefore, the information was from a very limited set of consumers. A basic tenet of sensory science is to clarify from which population a subsample is taken to understand the population to which it can be generalized (Stone and Sidel, 1985; Meilgaard *et al.*, 1991).

Various sensory evaluation techniques have been used in catfish research. Sensory tests have been used to quantify catfish nutrients (Nettleton *et al.*, 1990; Nettleton, 1990; Lovell, 1988), production yield and growth (Silva *et al.*, 1993; Webster *et al.*, 1993; Morris *et al.*, 1995) and processing storage stability (Boggess *et al.*, 1971; Tidwell, 1987; Silva *et al.*, 1993; Huang *et al.* 1992; Freeman and Hearnberger, 1994). Acceptability and preference scales have been used, but the participants have been small groups, and their demographics have not been described (Dupree, 1966; Dupree *et al.*, 1979; Lovell, 1983; Manthey *et al.*, 1988a; Huang *et al.*, 1992; Silva and Ammerman, 1993; Kim *et al.*, 1995). In the scope of sensory science, most of these evaluations would be considered screening of final products. They do not provide a general characterization of farm-raised catfish flavor.

Catfish acceptance data was cited within a survey of fish product flavors by Andrews and Grodner (1995), but depicted as unpublished. Their group consisted of 30 subjects, larger than previous studies but still less than the number of subjects recommended in consumer-type studies (Amerine *et al.*, 1965; Meilgaard *et al.*, 1991). In the publications using acceptance/preference testing, the extent to which the individuals were representative of a population of consumers has not been understood.

It is not well understood by demography which population prefers which combination of flavors (Chambers and Robel, 1993). A comprehensive study would focus on the basic catfish fillet or steak product and attempt to understand the range of overall desirability and acceptability. A large sampling of consumers would be required. Within the competitive aquaculture industry, consumer preference information may exist in a proprietary format. However, the information is not published.

To reduce the subjectivity of the word “desirable,” it may be defined as both: 1) a description of perceptions and statements about attributes by individuals, including their acceptability or preference; and 2) a description of the inherent, natural impact of flavor compounds within the food product (*e.g.* catfish). The focus in this work is on the latter, inherent attributes. Objective determination of innate attributes aims to examine their role in balance within the overall flavor impact of catfish.

In two investigations, semi-trained sensory methods were used to rate catfish by Dellenbarger *et al.* (1993) and Chambers and Robel (1993). Semi-trained means panelists would have been instructed in a few sessions to experience catfish attributes, but would not have received thorough training and practice. Neither sample set of participants in these two studies was large enough to allow generalization of the conclusions to even regional populations within the United States.

The most objective description of catfish flavor to date is from a sensory science research center. Chambers and Robel (1993) very carefully stated their panel’s description of three sets of farm-raised catfish from different growing

locations. Their multi-product panel described farm-raised channel catfish as having a mixture of low to moderate amplitude, white-meat, nutty/buttery and vegetative notes with sporadic muddy (decaying vegetation plus earthy) flavors. The muddy flavor was not in all fish, but could dominate the other flavors. Similar to the industry's description of catfish flavor impact (Johnsen *et al.*, 1987), this is the closest description in the literature of the overall flavor of farm-raised channel catfish. With the limited sample size, it still does not support a large generalization to a description of "characteristic" catfish flavor.

Sensory Evaluation in the Catfish Industry

Increased production in ponds has come at the cost of uneaten feed, heightening biomass levels, and the environmental off-flavor problem. Eliminating muddy/musty odors and flavors has led to quality evaluations for farm-raised fish. It is appropriate to make the off-flavor problem a priority because of the economic losses (Redmayne, 1989; Stickney, 1994).

Studying desirable and undesirable flavors and the balance of the two within catfish is prudent for marketability (Johnsen, 1989). Detection and threshold levels of geosmin and MIB have been determined (Lovell, 1983; Lovell *et al.*, 1986). The rate of uptake and depuration of the two compounds and resultant perception of off-flavors were demonstrated by Johnsen and Lloyd (1992). Extensive approaches to track the incidence of microbial effects on catfish growing conditions have included instrumental and flavor-by-mouth assessment (Bett and Johnsen, 1996).

Once the catfish industry had demonstrated economic stability and potential, research money was invested in helping the industry deal with perceived flavor problems. Collaborative research included businesses, trade organizations, universities and federal laboratories working toward greater understanding of the factors responsible for catfish flavor. To assess the status of flavor evaluation and to build a foundation upon which to work at that time, experienced sensory professionals guided a workshop of industry participants in defining catfish flavor attributes. This endeavor collected a lexicon (list of terms and definitions) of catfish flavors, both advantageous as well as those considered atypical and undesirable (Johnsen *et al.*, 1987). The generalization from this work concluded that the overall flavor perception of catfish is a low intensity blend of chicken-like, butter-like, vegetable-like notes that could easily be overcome by even part per trillion levels of some atypical, aromatic compounds (Johnsen *et al.*, 1987). The perception thresholds of these atypical compounds created an impression (or impact) far greater than their quantity should have indicated (Lovell *et al.*, 1986; Johnsen and Lloyd, 1992; Chambers and Robel, 1993). Because of the low levels of unwanted compounds, a trained sensory panel should perform quantitative assessment of differences from sample group to sample group.

Bringing the discrimination and descriptive ability of a quantitative sensory program to bear on flavor problems in aquaculture was helpful for progress. A trained descriptive analysis panel for catfish was established at the United States Department of Agriculture, Agricultural Research Service, Southern Regional Research Center

(USDA-ARS-SRRC), New Orleans, Louisiana, in 1988 (Johnsen and Kelly, 1990).

Some sensory evaluations performed focused on desirable flavor effects from 20 common feed ingredients (Johnsen and Dupree, 1991), the relationship of body fat content and off-flavor depuration (Johnsen and Lloyd, 1992), and a related study that assessed methods of perceiving and describing off-flavors (Johnsen and Bett, 1996).

Fat Content and Distribution within Fillets

Of primary concern in the research reported here is quantity of fillet fat. The quantity of fat is important in imparting flavor attributes to the catfish (Lindsay, 1985; Manthey *et al.* 1988a; Huang *et al.*, 1994; Morris *et al.*, 1995). The pattern of fat distribution within the fillet is influential in the impact of a balanced or unbalanced perception of fat in relation to other flavors (Manthey *et al.*, 1988a, Smith *et al.*, 1988; Johnsen and Kelly, 1990). In sensory evaluation techniques it is important that one subject does not receive a disproportionate quantity of fat in their sample.

Two interrelated total-lipid factors are dress-out percentages of the fish, which is a high priority for processors (Manthey *et al.*, 1988a; Silva *et al.*, 1993; Webster *et al.*, 1993; Conrad *et al.*, 1994; Robinson and Li, 1997), and refrigerated, iced and frozen storage stability, as mentioned above. In fundamental studies of the composition and distribution of body fat, Page and Andrews (1973) found that body fat increased as the digestible energy-to-protein ratio decreased. The exact percentage of protein that is advantageous in every situation has not yet been determined conclusively (Robinson and Li, 1997). Recent studies continue to elucidate catfish performance at various levels of protein in their diets. All of these factors relative to

fat content are of consequence in the second portion of this study, in which oil extraction from catfish fillets is a priority.

Dietary Needs of Catfish

Most nutritional requirements of channel catfish have been characterized, but a few are imprecise (National Research Council, 1983; Smith, 1989). The constraints of measuring feed intake in a watery environment hinder collection of data on specific requirements. Energy is the key life element needed for biological processes, waste metabolism and growth (Smith, 1989). Feeds should be formulated with prudence because channel catfish eat to satisfy their energy needs, possibly risking nutrient deficiencies (Page and Andrews, 1973; Gatlin and Stickney, 1982).

Dietary protein requirements for channel catfish range from 25 to 44%, depending on stage of life and fish size. Exact dietary protein requirement is influenced by feed allowance, water temperature, the energy sparing effect of other nutrients in the diet, and protein quality (Robinson, 1989). Growth may be restricted by a limiting quantity of any essential amino acid. Most base proteins of optimum composition in experimental fish diets are of animal origin. Casein is a complete protein for channel catfish, except a small deficiency in arginine. Muscle growth within the fish is a priority, while minimizing excess fat deposition caused by feeding too much dietary energy (Page and Andrews, 1973). Muscle weight is approximately 70% by dry weight in catfish (Manthey *et al.*, 1988a; Nettleton *et al.*, 1990).

The total lipid requirement in catfish diets for optimum weight gain has not been determined (Gatlin and Stickney, 1982; Robinson, 1989). Typically, diets

contain 5 to 6% lipid (Hardy, 1989). Lipids serve as a complementary source of energy that spares protein for tissue synthesis. In formulated feeds, lipids also support increased palatability and ease of pelleting. It has been found that moisture and protein content decrease in muscle tissue with increasing levels of dietary lipid (Stickney and Andrews, 1971, 1972; Page and Andrews, 1973; Dupree *et al.*, 1979).

Micronutrients are another category for which channel catfish requirements have not been fully established. Evidence that recommended levels of vitamins and minerals promote growth and prevent mortality have been published in several sources (National Research Council, 1983; Robinson, 1989; Wilson, 1991; Stickney, 1994). Channel catfish do not require carbohydrates in the diet, however, they can be metabolized (Robinson, 1989). Carbohydrates provide low cost, protein-sparing energy in diet formulations.

General Methods of Sensory Evaluation using Descriptive Analysis (DA)

The process of establishing a DA panel combines: 1) building panelists' experience with the actual product, 2) creating activities with references that clearly show individual attributes of the product, and 3) learning a rating or measuring scale to describe intensity within the product and of the references. The intensity rating is a numerical score that can be calculated for sample statistics. The rating may be on a category or a continuous scale. Training and practice of a rating system makes the method objective.

Three basic DA systems are common in sensory evaluation: Quantitative Descriptive Analysis™, the Flavor Profile, and the Spectrum™ universal intensity

scale. Within each system, a set of essential practices differentiates the method's application from the others. All emphasize the use of preplanned experimental design and rely on the use of a group of trained subjects.

In Quantitative Descriptive Analysis™ (QDA), initiated by Stone and Sidel (1985), one basic tenet is that panelists be slightly familiar with the product to be evaluated. An unmarked, 15-centimeter line scale with anchored terms or products at each end is used. Measurement in millimeters from the left end of the line provides a scale, but each panelist is permitted to choose the level of meaning of the characteristic on the line, with one line per attribute. This method is commonly used with as many attributes as needed. A second tenet is that all rating lines describe product specific attributes, not attributes that could occur in any product within that class.

The Flavor Profile method, the earliest DA method (Caul, 1957), utilizes a set of symbols that denote a seven increment scale of increasing value. The scale is taught to panelists using sets of basic taste solutions (*sweet, salty, sour and bitter*) at specific concentrations. The symbolic scale is learned by practicing with the reference solutions, and abstracting the intensity levels to products. An important tenet of this method is that the panel leader reports the panel's consensus value for each product attribute. The consensus is accomplished by collecting each panelist's scores when all are present at a group session. The purpose of this group discussion session is for panelists to inform each other of their evaluation and for each to convince the others that their evaluation is the most correct. A final score is not registered until the group

decides on their consensus. The mutual influence and non-statistical basis of this method renders it less useful for true descriptive analysis.

The Spectrum™ method of universal intensity for sensory evaluation (Meilgaard *et al.*, 1991) is aptly named because, theoretically, the intensity scale is infinite. Training within the intensity scale is concentrated in the range where most products are likely to fall, *i.e.* at levels 0-15. Catfish flavor attributes are low in intensity, so the bottom portion of the flavor scale was used. But the system can be modified by adding more intense references to the upper limit or by concentrating within one range of the scale and adding more specific references to define more precise differences. The intensity references are common products and methodologies are published for all five human senses, *e.g.* skinfeel or food texture (touch), basic taste (taste), appearance (sight), sound (hearing) and odor (smell). The scales are theoretically boundless. The set of references (Meilgaard *et al.*, 1991) used for the flavor descriptive analyses appear in Table 1.

The recommended procedures (Meilgaard *et al.*, 1991) are flexible enough to add more references to train panelists, and guidelines are published for evaluation by any of the five senses. The useful trait of the Spectrum™ scale for taste or texture, for instance, is that once learned, one can rate a new sample by the same measurements without any other assistance. These measurements are then discerned within a context of other products, and clearer comparisons are made. Also, panelists can communicate to each other more clearly when switching to a different product. Practice and experience with the measurements (*i.e.*, the reference products) create a context

Table 1. Intensity references for flavor attributes using the Spectrum™ method.

Intensity	Flavor Descriptor	Reference Product
1	cooked wheat	Wheat Thins (Nabisco Brands, Inc.)
2	oil flavor	Lays Potato Chips (Frito-Lay, Inc.)
3	buttery	Land-O-Lakes Margarine (Land O'Lakes, Inc.)
4	grape	Grape Kool-Aid (General Foods)Corp.)
5	apple	Mott's Natural Apple Sauce (Mott's USA, Cadbury Schweppes, Inc.)
6		
7	orange complex	Minute Maid Frozen Concentrated Orange Juice (Coca-cola Foods)
8		
9		
10	grape	Welch's Grape Juice (Welch's)

of general flavor intensity by which a newly experienced product can be understood. The resulting measurements constitute an ordinal, continuous scale of intensity levels that can be statistically calculated. Depending on the level of training and practice received, the Spectrum™ universal method can be used by experienced panelists for any kind of product screening or by more highly trained panelists to provide descriptive analysis.

The Spectrum™ method was chosen for the USDA-ARS-SRRC flavor panel (Johnsen and Kelly, 1990) to provide a research tool that expanded the observations made by industry representatives (Johnsen *et al.*, 1987). The Spectrum™ method was chosen because of its ability to communicate perceptions at low intensities and the adaptability of the rating scale for use with attributes that were not on the pre-set ballot. All panelists were trained in the method and given maintenance sessions periodically to confirm their sensory memory of the reference intensities and attribute flavors. New members were trained and integrated into the panel as necessary to keep a minimum number of 15 panelists. The majority of the research projects in which the panel participated were experiments to judge the presence or absence of environmentally induced flavors. The fact that the discriminatory ability of the trained panel was needed for the industry's troublesome affinity for 2-methylisoborneol and geosmin has been documented (Johnsen, 1989). Progress and conclusions drawn in these endeavors have been discussed by Bett and Johnsen (1996).

General Methods of Gas Chromatography-Olfactometry

Analytical approaches to detecting food flavors have used gas chromatography (GC) since its introduction in the 1950s (Hartman *et al.*, 1993). Many combinations of preparation techniques and GC instrument attachments have been devised to improve the extraction, separation and identification of compounds. Since the food materials usually change during storage, continued improvements in methodological approaches are necessary (Reineccius, 1993). Researchers have aimed to sample the food by matching the state in which it would be consumed. Generally, though, only a fraction of the volatile and non-volatile components that make up flavor are captured.

Using preparative and separation GC techniques, flavor analyses determine a subset of what is detected by human sensations. The method focuses on volatile components extractable by solvent or conducive to escape into the surrounding headspace. The volatiles would normally be in the food matrix along with other molecules that impart flavor, *e.g.* peptides or disaccharides. The pure number of GC peaks seen on a chromatogram are not as clear a “snapshot” of flavor as they appear. Techniques have been further improved to consider the contribution of the detected volatiles to flavor impact and the characteristic nature of the volatiles (Reineccius, 1993). The relationship of instrumental detection methods to the sensory properties of the food is essential (Pollien *et al.*, 1997). Improved methods aim to detect what is important and accurate about the foodstuff (da Silva *et al.*, 1994; Pollien *et al.*, 1997). Then, interpretation of the data from accurate methods leads from “detection only” to a beginning understanding of the impact of flavor compounds on the food.

An advanced technique devised to use simultaneous sniffing methods with GC detection events is called gas chromatography-olfactometry (GCO). Within several variations of this method, the human nose plays the role of a second detector at the same time the effluent is passing the GC detector. Olfactometric (or “sniffing”) techniques allow the determination of “impact odorants” in foods. A definition of GCO, as stated by da Silva *et al.* (1994) is:

“GCO’s use in flavor research has three objectives: (1) to establish odor-active compounds in flavor extracts, (2) to determine a compound’s single odor quality, and (3) to quantify a compound’s individual odor significance in flavor systems

GCO techniques can be classified into two categories. The first category, dilution is perceived at the GC sniffing port. The second category, the intensity-type method, includes techniques in which the aroma extract is injected once while the panelist records the odor intensity as a function of time. The time element may be documented by a hand-held device (like a joy-stick) or by voice recording using a tape recorder or by a human transcriber.

Dilution-type methods are most often cited in GCO literature. The two modes are Charm analysis (Acree *et al.*, 1984) and aroma extract dilution analysis (AEDA) (Ullrich and Grosch, 1987). Both techniques integrate the use of threshold values of odorants (volatiles) to calculate theoretical impact on the total impression of the food (Grosch, 1994).

One important intensity-type method, Osme, uses a computer to record the duration and intensity of the volatiles emitted as GC effluent (da Silva *et al.*, 1994).

The objective is to achieve a psychophysical estimation of the individual odor intensity. This laboratory's investigation of predetermined mixtures has tested panelist training and terminology, panelist performance variation, and several methods of statistical analyses (da Silva *et al.*, 1994). Many techniques are a hybridization of these three, depending on the resources available and the perishability of the foodstuff.

The rate of discovery in GCO techniques is rapid at this time. Several authors (Reineccius, 1993; Pollien *et al.*, 1997) have reviewed the approaches. Investigations in some facilities scrutinize the method as well as the food product (Abbott *et al.*, 1993a, 1993b; da Silva *et al.*, 1994; van Ruth *et al.*, 1994). Prudent judgment in drawing conclusions must be based on understanding the quantity and source of panelist variability (Abbott *et al.*, 1993b). Reineccius (1993) emphasized the importance of understanding from what portion of the total food the subsample is taken (*e.g.* whole tissue, water or soluble fraction, *etc.*) and the type of extraction (*e.g.* headspace analysis, purge and trap, direct injection, *etc.*).

Synopsis

The current knowledge of catfish flavor is a blend of empirical data and anecdotal opinions. From these, one gets a sense of the range of characteristic flavors. Overall flavor impact was best described by a trained multi-product panel (Chambers and Robel, 1993) as a combination of low to moderate amplitude, white-meat, nutty/buttery and vegetative notes. We speculated that these are the most favorable traits of catfish. The sporadic decaying vegetation and earthy flavors noted by the panel had the ability to dominate the other flavors. The latter flavors impact the

industry significantly, but discussion of them falls outside the scope of this investigation.

Clear marketing facts have not been gathered about the impact of typical catfish flavors on consumers and their purchase decisions. The timing of this study was opportune to employ the trained descriptive flavor panel at USDA-ARS-SRRC. The study was planned to further determine data on desirable flavors in farm-raised catfish. Positive effects of an earlier time-on-diet experiment implied that more work on these variables would be beneficial. Opportunities to grow the fish indoors were also available; this reduced the interaction of environmental factors and helped elucidate the influence of feed formulation and genetics on farm-raised catfish flavors.

CHAPTER 3

DESCRIPTIVE FLAVOR ANALYSIS OF FARM-RAISED CATFISH -- FEED FORMULATION, TIME-ON-DIET AND GENETIC STRAIN EFFECTS

Introduction

Marketing demand for farm-raised seafood products has continued to increase because of safety, availability and perceived quality (Johnsen, 1991). Flavor characteristics of commercial fish products are also important quality factors for consumer acceptance (Wesson *et al.*, 1979; Sawyer *et al.*, 1988). In United States aquaculture enterprises, channel catfish (*Ictalurus punctatus*) exceeds all other species in quantity produced (Redmayne, 1989). The catfish industry harvested over 215, 000 metric tons valued at \$365 million dollars in 1996 (Anonymous, 1996). While farming and processing practices have improved steadily, marketing has been restrained because of environmentally induced, muddy/musty off-flavors (Johnsen, 1989; Bett and Johnsen, 1996). Most flavor research has been focused on these off-flavors (Robinson, 1989; Stickney, 1994). The appealing, desirable attributes of channel catfish that provide the underlying flavor matrix to balance these off-flavors have not been fully characterized (Johnsen *et al.*, 1987; Chambers and Robel, 1993).

Current knowledge of desirable catfish flavors has employed a range of sensory methodologies. Early flavor profiles of channel catfish described changes due to pond condition, seasonal and storage effects (Maligalig *et al.*, 1973; 1975a; 1975b). These evaluations were by a semi-trained panel. In many catfish production and storage studies, acceptance by a small panel has been published using hedonic scales

(Dupree, 1966; Dupree *et al.*, 1979; Lovell *et al.*, 1986). An experienced semi-trained panel compared catfish species (channel and two European strains) using an acceptability scale (Manthey *et al.*, 1988a). Alternate protein sources in catfish feed formulations have been rated by triangle tests (Conrad *et al.*, 1994) and trained descriptive panels (Johnsen and Dupree, 1991; Webster *et al.*, 1993). These reports provide sporadic information on catfish flavor impact. The expected range of desirable flavors in a catfish product still has not been described.

Understanding the degree to which innate catfish constituents contribute to its overall flavor impact is useful. Published research during early growth of the industry used simple flavor techniques without planning sensory experimental designs. At the same time, sensorial evaluations were becoming more standardized. These standards improved product descriptions. In the catfish industry, structured sensory evaluations of production and storage issues have become common tools to assess product quality. Feed ingredient substitutions have not significantly altered the flavor of channel catfish (Johnsen and Dupree, 1991; Webster *et al.*, 1993; Conrad *et al.*, 1994). These selected ingredients were used to test seasonal, low-price nutrients that maintain fish growth to provide least-cost feed formulation. Flavor intensity scores reported in these studies gave detailed information on treatment differences, but not descriptive profiles. Descriptive knowledge of innate components may lead to hypotheses of proper balance between desirable and undesirable flavors in final products. If production treatments can have a highly positive effect on desirable attributes they may render off-flavors less perceivable.

A range of channel catfish flavors was described in a survey of freshwater fishery products' flavors. Chambers and Robel (1993) reported the most comprehensive summary of catfish flavor impact to date. Their trained, multi-product panel described farm-raised channel catfish as having a mixture of low-to-moderate amplitude white-meat, nutty/buttery and vegetative notes with sporadic muddy (decaying vegetation plus earthy) flavors. The muddy flavor was not in all fish, but it could dominate the other flavors. Samples represented three areas from the southern United States growing region. Although it covered a limited number of sampling locations, this description captures the essence of catfish flavor most appropriately to date.

Some desirable flavor research has focused on feed ingredient substitution. A subset of catfish flavor attributes was used to determine if 20 common feed ingredients influenced desirable flavors (Johnsen and Dupree, 1991). Flavor intensities from the feed ingredients were compared to those of a casein-based, semi-purified diet. Few differences were found due to feed ingredient in these 150g fish. Grow-out size was smaller than average market catfish, and intensities of the five flavors were not organized together to show total flavor impact which might be generalized to typical, desirable catfish flavor. An observation made during the 20-ingredient study was that the reference, casein-based catfish on feed for 300 days had significantly higher intensities of the five desirable flavors studied.

Following this observation, the objectives in this study were to further test four of the preceding feed ingredients that had exhibited the largest flavor intensity

differences in three or more desirable attributes (Johnsen and Dupree, 1991). Divided into two studies, we first investigated the influence of type of ingredient, fish size (or time-on-diet), and fillet fat content on desirable flavors. A second experiment compared catfish genetic strain flavor differences. Trained panel descriptive analyses and triangle test difference tests were used to evaluate differences.

Materials and Methods

A. Husbandry

1. Study I: Feed Effects

Juvenile channel catfish (*Ictalurus punctatus*) from one spawning were obtained from a commercial supplier. Catfish fry were grown in aluminum troughs on commercial catfish feed formulation at the United States Fish and Wildlife Service, Fish Farming Experimental Laboratory, Stuttgart, Arkansas, according to the method of Johnsen and Dupree (1991). The start date that fry were placed on experimental diet was modified from the previous method because of a delay to treat a bacterial infection with furasone and oxytetracycline (Terramycin). Catfish fingerlings of mode weight, 97.5g (previous beginning weight 75g), were started on experimental diets on June 11, 1991.

Fingerlings were randomly assigned to indoor fiberglass culture tanks (1.52 m diameter x 0.61 m deep) with a water capacity of 266 L and were supplied with single-pass heated (27°C) well water at the rate of 20 L per minute. Each tank housed fish on one experimental diet (semi-purified or commercial plus carboxymethylcellulose

binder). Sample codes (Table 2) were assigned to experimental diets described in Table 3. Two tanks were required to grow enough catfish for the REF_CASE diet to meet sensory panel material needs. Experimental fish were monitored to provide a population of live fish within a certain weight range. Rate of feeding was provided by a conveyor system above each tank and modified accordingly to allow approximately 10 pellets to remain after group feeding.

The PRAC diet group showed aggressive behavior and killed most of the fish in their tank. The replacement fish placed on PRAC diet were no longer from the same spawning. The culture tank for PRAC was restocked after Harvest 2 with typical, young-of-the-year previously raised on commercially-formulated feeds for catfish and maintained in tanks supplied with single-pass well water. The fish size-range was selected to match the size of the other groups of experimental fish. At the time of Harvest 4, a shortage of PRAC group catfish flesh required that REF_CASE fillets be substituted for PRAC in the triangle tests only.

2. Study II: Genetic Effects

Five genetic strains of catfish were chosen from stocks at the United States Department of Agriculture, Agricultural Research Service, Catfish Genetics Research Laboratory, Stoneville, Mississippi. Sample codes (Table 4) identified the genetic strains.

Catfish were grown in indoor circular culture tanks (1.52 m diameter x 1.22 m deep) with a water capacity of 662 L and were supplied with heated (26°C),

Table 2. Designated codes for channel catfish feed formulations in Study I, Feed Effects.

CODE	DESCRIPTION
REF_CASE	Reference, Casein-base feed
PRAC	Practical, a commercial feed formulation
CFML	10% Catfish Meal feed
MTBN	10% Meat and Bone Meal feed
RICE	10% Rice Bran feed
MOIL	3% Menhaden Oil feed

Table 3. Composition (g/kg) of semi-purified feed formulations used in Study I, Feed Effects.

<u>Code</u>	<u>Description</u>	<u>Test Ingredient</u>	<u>Casein</u>	<u>Dextrin</u>	<u>Vegeta- ble Oil</u>	<u>Cellulose</u>	<u>Other¹</u>
REF_ CASE	REFERENCE, CASEIN		420	150	60	270	100
PRAC	PRACTICAL,	920	0	0	0	0	80
	commercial catfish feed						
CFML	CATFISH MEAL,	100	361	150	50	239	100
	processing plant renderings,						
	57% crude protein						
MTBN	MEAT AND BONE MEAL,	100	368	140	51	241	100
	rendered,						
	50.4% crude protein						
RICE	RICE BRAN,	200	395	49	33	223	100
	12.7% crude protein						
MOIL	MENHADEN OIL	60	420	150	0	270	100

¹Other ingredients in g/kg dry diet: 50.0 g carboxymethylcellulose; 20.0g salt mixture USP XIV; and 30.0 g vitamin mixture. Salt mixture USP XIV contains in g/kg: ammonium alum – 0.092; cupric sulfate – 0.078; ferric ammonium citrate – 15.29; manganese sulfate – 0.201; potassium iodide – 0.041; sodium flouride – 0.507; calcium carbonate – 68.6; calcium citrate 308.3; calcium biphosphate – 112.8; magnesium carbonate – 35.2; magnesium sulfate – 38.3; potassium chloride – 124.7; dibasic potassium phosphate – 218.8; and sodium choloxide – 77.1. Vitamin mixture contains for each kg: vitamin A palmitate – 5000 IU, calciferol – 4800 IU; alpha tocopherol acetate – 60 IU; menadione – 20 mg; ascorbic acid – 500 mg; thiamin – 50 mg; riboflavin – 100 mg; pyridoxine – 50 mg; pantothenic acid – 200 mg; nicotinic acid – 750 mg; biotin – 5 mg; folic acid – 25 mg; vitamin B-12 – 0.1 mg; choline – 15 g; inositol – 2 g; and non-nutritive bulk (filler) – 11.240.

recirculating well water. Fish were fed a commercial catfish fingerling diet (Table 5) manufactured by MFC Services, Madison, Mississippi. The BULL group had been captured from local streams. Fourteen days before harvest, BULL and REF_CHAN_FARM (described below) were placed in similar indoor tanks and fed the commercial fingerling diet.

As a reference for each replication of panel sessions, typical channel catfish fillets were chosen to provide a sixth, representative group. In Harvest 1, fillets were purchased from a local commercial retailer (REF_CHAN_COMM). In Harvest 2, catfish fillets were acquired from Delta Branch Experimental Station (REF_CHAN_FARM) to duplicate a level of geosmin/MIB intensity that was perceived (but not intended) in Harvest 1.

In Harvest 2, SLOW (channel catfish) was substituted for BULL in the triangle tests because of a shortage of BULL group catfish.

B. Harvest and Processing of Catfish

Fish were processed according to practices described in Johnsen and Dupree (1991). After harvest, eviscerated catfish rounds were frozen at -20°C for 1-5 days until sample preparation. To prepare samples, the rounds were thawed in an 8 to 10°C water bath in their storage bags. A Jaccard Model A35-P membrane skinner (Orchard Park, NY) accomplished skinning. The crucial, subcutaneous layer was preserved (Johnsen and Dupree, 1991). Shank fillets were prepared by hand. The final preparation was Blended Individual Fish Samples (BIFS) for all sensory sessions and

Table 4. Designated codes for genetic groups used in Study II, Genetic Effects.

CODE	DESCRIPTION
FAST	channel catfish “fast-growing”, <i>Ictalurus punctatus</i> Kansas x Kansas
ALBI	channel catfish, albino, <i>Ictalurus punctatus</i> Mississippi Albino x Mississippi Albino
BLUE	blue catfish, <i>Ictalurus furcatus</i> Blue x Blue
BULL	black bullhead catfish, <i>Ameiurus melas</i>
HYBR	hybrid catfish, channel <i>Ictalurus punctatus</i> Red River x blue <i>Ictalurus furcatus</i> Blue
REF_CHAN_ COMM	Harvest 1, Reference, Channel Catfish purchased at a local retail fish store
REF_CHAN_ FARM	Harvest 2, Reference, Channel Catfish from Delta Branch Experiment Station, Mississippi State University, Leland, Mississippi
SLOW	channel catfish “slow-growing” <i>Ictalurus punctatus</i> Mississippi Normal x Mississippi Normal

Table 5. Composition of feed formulation used at Catfish Genetics Research Laboratory in Study II, Genetic Effects.

FINGERLING CATFISH FOOD¹

CRUDE PROTEIN	NOT LESS THAN 35.0%
CRUDE FAT	NOT LESS THAN 2.5%
CRUDE FIBER	NOT MORE THAN 6.0%
MOISTURE	NOT MORE THAN 12.0%

INGREDIENTS:	Niacin Supplement	Dicalcium Phosphate
Soybean meal	Vitamin B12 Supplement	Traces of:
Fish Meal	Choline Chloride	Manganous Oxide
Ground Corn	Menadione Sodium Bisulfite	Calcium Iodate
Ground Wheat	Thiamine Mononitrate	Copper Oxide
Vitamin A Supplement	Ascorbic Acid	Cobalt Carbonate
Vitamin D3	Pyridoxine Hydrochloride	Zinc Oxide
Supplement	Folic Acid	Iron Carbonate
Vitamin E Supplement	Ethoxyquin A Preservative	Sodium Selenite
Riboflavin Supplement	Salt	
Calcium Pantothenate		

¹Manufactured by MFC Services, Madison, Mississippi

bulk packages for chemical analyses (Appendix A). Unique four-digit codes were pre-labeled on the BIFS boiling pouches for all sessions of sensory evaluation.

C. Selected Body Composition Measurements

Total fillet fat was determined by a modification of the chloroform-methanol method of Koniecko (1979). Moisture was determined by drying samples to a constant weight at 100-102°C during 16-18 hours (AOAC, 1990). Percent fillet fat was then calculated on a dry weight basis for reports.

D. Sensory Experimental Design by Power Analysis

We speculated that time-on-diet had a positive effect on desirable flavor intensity. In the 1991 investigation of 20 feed ingredients, higher scores were found for the 300-day Reference-Casein catfish (Johnsen and Dupree, 1991). With this evidence, a procedure of hypothesis testing to set a minimum sensory-score magnitude of difference was completed (Zar, 1981). After the previous study, more specific interpretation of small statistical differences on the Spectrum™ scale was desired to understand their true meaning.

In stepwise fashion (SAS Inc., 1985), the panel-score variances for the Reference-Casein catfish at Time 0 Days and Time 300 days were calculated to determine average variances (Johnsen and Dupree, 1991). An example portion of the programming is shown in Appendix B. The average variance was plugged into an equation that was resubmitted with each of the power levels, with β error to be considered at 80, 85, 90 or 95 percent. Alpha error, α , was constant at 0.05. The numbers generated indicated a Minimum Detectable Difference (MDD) necessary to

conclude two treatments were truly different. The difference would be based on the Spectrum™ evaluation method, in which the flavor panel was trained (as described below, Meilgaard *et al.*, 1991). The results were listed separately for each level of power, and from that a judgment was made to select an appropriate MDD. As a matter of quality control, the selected MDDs for each attribute were planned to represent a level of discrimination that a consumer realistically could not detect (personal communication, Gail Vance Civile).

E. Sensory Descriptive Analysis

A panel of 8 to 12 trained judges per session performed descriptive flavor analysis. The panel consisted of 7 females and 5 males ranging in age from 20 to 75 years that had served on the panel from 14–40 months. The Spectrum™ method of intensity rating (Meilgaard *et al.*, 1991) was used to train the panelists with the 16 descriptors previously developed for catfish (Johnsen *et al.*, 1987; Johnsen and Kelly, 1990).

Maintenance of trained skills was accomplished by intermittent panel sessions focusing on evaluation of concept and scaling samples. All descriptors were scored by the panelists to eliminate the need to give instructions that would yield psychological bias. Five desirable attributes, *Chickeny*, *Nutty*, *Fat Complex*, *Corn*, and *Sweet*, were further analyzed for this experiment. Definitions in Table 6 denote the reference materials used to train judges to describe each flavor perception. The CompuSense software system (CompuSense, Inc., Guelph, Ontario, Canada) was used for data collection.

Table 6. Catfish desirable flavors used in experimental analyses.

TERM	DESCRIPTION
<i>Chickeny</i>	The aromatic associated with sweet cooked chicken meat.
<i>Nutty</i>	The aromatic associated with fresh pecans and other hardshell nuts.
<i>Fat Complex</i>	The aromatic associated with dairy lipid products, melted vegetable shortening, and cooked chicken skin.
<i>Corn</i>	The aromatic associated with cooked corn kernels.
<i>Sweet</i>	The taste on the tongue associated with sugars.

Samples were presented under red light to the panelists for flavor-by-mouth assessment. Each descriptive panel session began with panelists reviewing the intensity references (Chapter 2, Table 1) followed by tasting one “typical” farm-raised catfish sample. The scores for this sample were not included in the main study statistical analyses. This activity is termed a “warm-up” and is used to promote calibration of each panelist during that session (O’Mahony *et al.*, 1988). Panel means for each attribute of the “warmup” catfish were calculated by CompuSense and immediately discussed by the group for that session’s panel calibration.

Samples were fully randomized and presented at 7-minute intervals. Unsalted crackers and distilled, deionized, room temperature water were used to rinse the mouth between samples. All six experimental treatments were evaluated in each session of Study I and II, and each treatment was presented five times for descriptive analyses.

The sensory data were analyzed with the GLM procedure of SAS (SAS Inc., 1985). A split-plot design was used to test for feed ingredient effects (Study I) because catfish were drawn from the same treatment tank. The whole plot tested was replicate and subplot was feed treatment. A one-way analysis of variance was used to test the completely randomized design of genetic samples (Study II). If significant differences were found, Tukey’s significant difference test was applied (Appendix C).

F. Triangle Tests

Judges could participate in triangle test evaluations only if they had received procedural training (Meilgaard *et al.*, 1991). A group of 27 judges was acquainted with the test procedure using a practice test of two orange juices. Conventional

triangle tests were conducted according to Larmond (1977) and Roessler *et al.* (1978). Eighteen judges from the pool of 27 completed a randomized scheme comparing all treatments for each harvest. Results were analyzed by the binomial method of Roessler *et al.* (1978).

In two cases, substitution of alternate catfish product was made in triangle tests because of the unavailability of the original treatment. In the Feed Effects study, catfish from the REF_CASE group replaced the PRAC feed catfish in Harvest 4. In the Genetic Effects study Harvest 2, BULL catfish were replaced by SLOW channel catfish that had been fed the same catfish fingerling feed.

Results and Discussion

A. Husbandry and Fillet Fat Content

Catfish harvests in the Feed Effects study occurred at approximate market weights of 1/3, 1, 2 and 3 pounds (mean live weights, 164.9g, 465.6g, 912.0g and 1501.5g, respectively, Table 7). Average fillet yields of 32.65-33.23% of live weight were lower than found by other researchers (Conrad *et al.*, 1994; Huang *et al.*, 1994; Robinson and Li, 1997). This was expected because the filleting procedure in this investigation was not done quantitatively. Increasing amounts of visceral fat were observed as time-on-diet increased, but they were not measured. Visceral fat quantity is a consideration for producers who desire to minimize by-product waste. These fish were grown in constantly warm water (27°C) that has been reported to increase growth rate (Stickney and Andrews, 1971) and may have increased the rate of deposition of fat.

Table 7. Mean weights (g) of channel catfish in Study I, Feed Effects.

DIET ¹	HARV	Range of live weights in group		Round wt	Fillet wt per 2 fillets	Avg Yield % Fillet/live wt
		MIN - MAX	Mean	Mean	Mean	
REF_CASE	1	111-194 ²	153.4 ²	99.7	50.4	32.86 ²
	2	413-513	468.8	300.2	149.1	31.81
	3	829-1011	912.0	593.1	307.6	33.75
	4	1204-1991	1579.9	981.2	477.6	30.39
PRAC	1	118-191 ²	159.8 ²	103.9	52.9	33.10 ²
	2	254-467	376.2	251.8	133.1	35.39
	3	703-912	850.9	558.6	292.2	34.36
	4	524-1375	1108.5	740.8	387.6	34.96
CFML	1	125-197 ²	164.8 ²	107.1	54.8	33.25 ²
	2	417-630	525.2	343.9	173.1	32.96
	3	884-980	935.9	601.1	304.1	32.52
	4	148-2043	1682.0	1068.8	525.0	31.04
MTBN	1	138-206 ²	175.4 ²	114.0	56.6	32.27 ²
	2	447-541	492.1	321.0	162.2	32.97
	3	856-929	902.0	581.7	294.3	32.63
	4	1178-1670	1460.0	957.4	482.8	33.13
RICE	1	129-195 ²	167.7 ²	109.0	54.4	32.44 ²
	2	LD ³	LD ³	LD ³	LD ³	LD ³
	3	879-1034	930.4	604.8	304.4	32.73
	4	971-1834	1541.1	997.9	511.0	33.16
MOIL	1	115-202 ²	168.3 ²	109.4	54.9	32.62 ²
	2	LD ³	LD ³	LD ³	LD ³	LD ³
	3	884-1020	941.0	609.3	314.4	33.41
	4	1274-2016	1637.7	1085.1	544.0	33.22
AVG HARVEST1		112.7-197.5 ²	164.9 ²	107.2	54.0	32.76 ²
AVG HARVEST2		382.8-537.8	465.6	304.2	154.4	33.28
AVG HARVEST3		839.2-981.0	912.0	591.4	302.8	33.23
AVG HARVEST4		1105.7-1821.5	1501.5	971.9	488.0	32.65

¹REF_CASE = Reference, Casein-base feed

PRAC = commercial catfish feed

CFML = 10% catfish meal feed

MTBN = 10% meat and bone meal feed

RICE = 10% rice bran feed

MOIL = 3% menhaden oil feed

²Estimated from round weights and multiplied by 65% (average) yield³LD = lost data

As expected, average fillet fat (dry-weight basis) in the Feed Effects study increased with time-on-diet for each feed formulation (Figure 1). Fillet fat is important in product storage, eating quality and heart-healthy nutrition concerns. After 310 days on-diet and the maximum live weight category, about 1500g (3 pounds), the fillet fat content of 7.36% is still desirable for consumption as a heart-healthy food compared to some non-fishery protein products.

Results of two replicate harvests of selected catfish strains in Study II are shown in Table 8, followed by results of fillet fat analyses in Figure 2. The fillet fat contents were lower in the second harvest. A rationale for this effect cannot be offered since the catfish were grown under the same indoor conditions and neither harvest (July or October) occurred during periods of low activity (Stickney, 1994).

Huang *et al.* (1994) reported fillet fat content of channel and channel x blue hybrid catfish as 5.0% and 5.5% in wet tissue, respectively. Our mean fat results in both harvests were similar for FAST channel group at 5.80% and 3.56% wet basis, respectively. Fillet fat in HYBR hybrid catfish was lower in both harvests, 4.17% and 2.57% wet basis, respectively, than results found by Huang *et al.* (1994). Most hybrid catfish studies reviewed in this article had as their aim to collect pond production information rather than eating characteristics. Huang *et al.* (1994) and our results appear to be the extent of information on fillet quality of hybrid catfish at this time.

Comparing catfish genetic strains during processing, one additional observation from the sample preparation phase was the difficulty in ensuring that the mechanical skinner removed all skin of the ALBI albino channel catfish. The color of

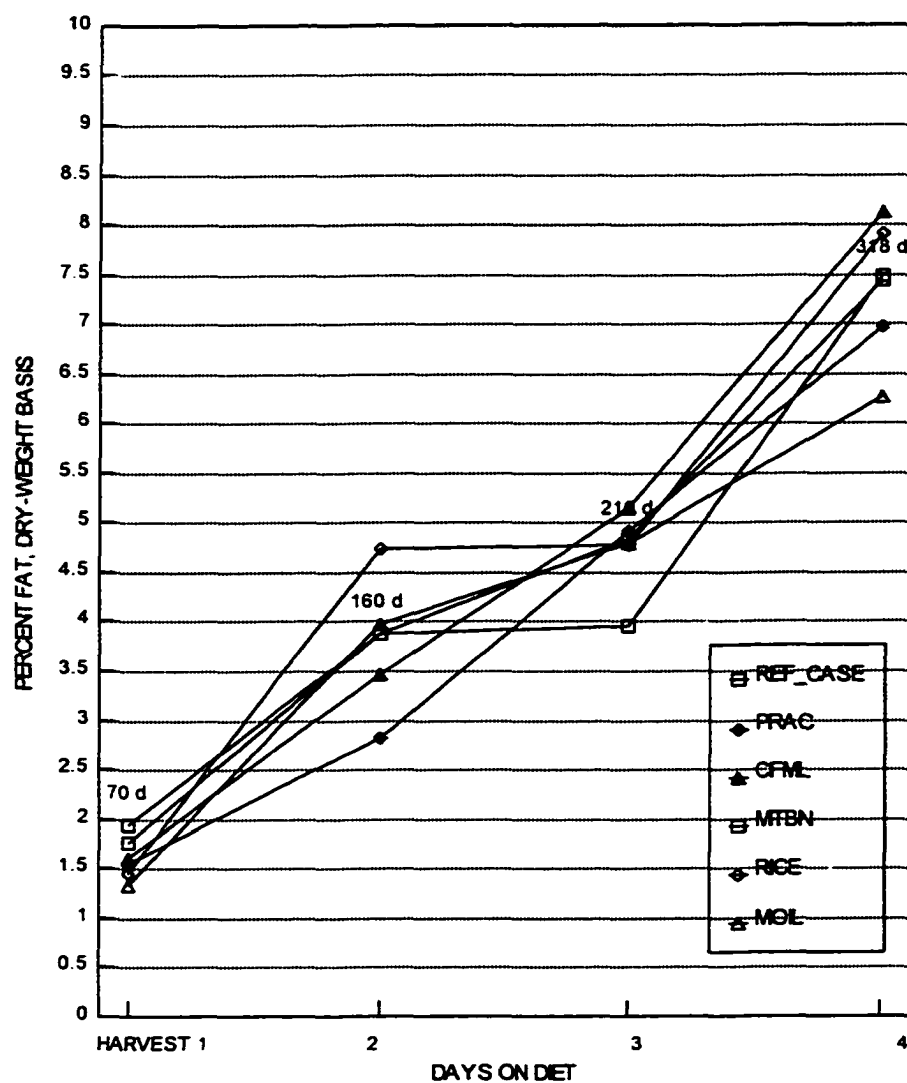


Figure 1. Mean percent fillet fat (dry weight basis) in channel catfish fed Casein-based formulations in Study I, Feed Effects.

Table 8. Mean weights (g) of catfish in Study II. Genetic Effects.

GENETIC STRAIN ¹	HARV	Range of live weights in group		Round wt	Fillet wt per 2 fillets	AvgYield %
		MIN - MAX	Mean	Mean	Mean	Fillet/live wt
FAST	1	362-705	475.3	311.7	161.2	33.91
	2	273-820	579.8	354.8	158.4	27.51
ALBI	1	246-474	357.4	226.7	119.2	33.35
	2	147-482	297.6	188.6	78.2	26.36
BLUE	1	144-328	208.0	132.2	67.9	32.62
	2	104-508	268.7	168.7	77.4	28.61
BULL	1	63-202	106.6	57.9	27.6	25.86
	2	47-123	70.3	36.7	14.7	20.63
HYBR	1	294-638	453.4	290.2	155.1	34.21
	2	152-1209	405.9	251.1	115.1	28.79
REF_CHAN_COMM ²	1				362-400	
REF_CHAN_FARM	2	372-1164	623.1	354.3	178.3	28.82
AVG HARVEST1		222-320.1	320.1	203.7	106.2	31.99
AVG HARVEST2		183-374.2	324.5	225.7	103.7	27.71

¹FAST = channel catfish, fast growing

ALBI = albino channel catfish

BLUE = blue catfish

BULL = black bullhead catfish

HYBR = hybrid channel x blue catfish

REF_CHAN_COMM = commercial retail store channel catfish

REF_CHAN_FARM = farm-supplied channel catfish

²Harvest data not determined because channel catfish fillets were purchased from a retail supplier.

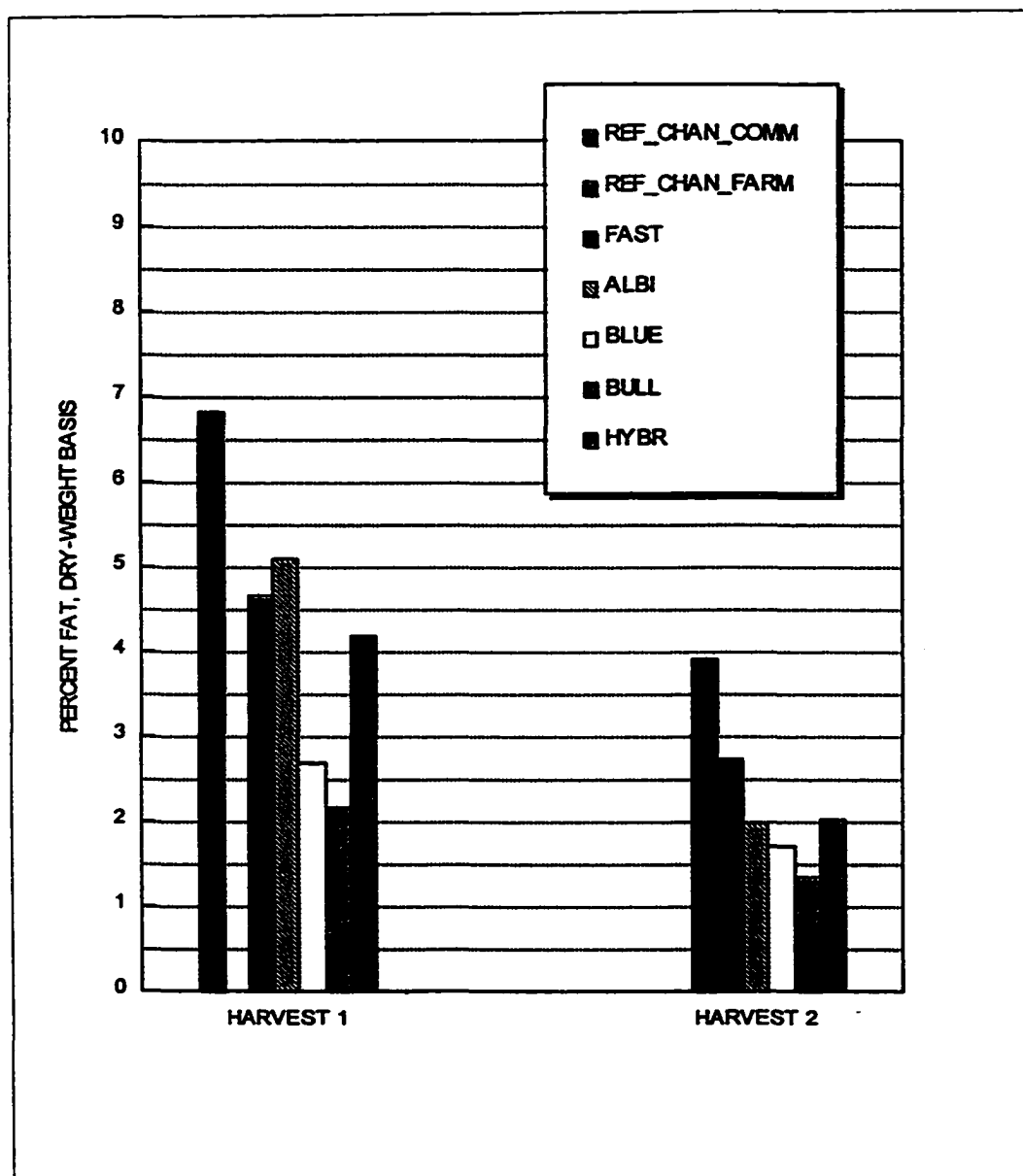


Figure 2. Mean percent fillet fat (dry weight basis) in strains of catfish in Study II, Genetic Effects.

the skin is so similar to the underlying fascia and flesh that extra time is needed to ensure that skin does not remain on the fish rounds before filleting.

B. Power Analysis and Statistical Approaches

To decide on the number of panel descriptive analysis (DA) replicates, the results of the power analysis calculations were used to judge a reasonable level of error. The aim was to achieve a balance of: 1) sensitivity of objective sensory panel scores with 2) the manpower costs of panelist fatigue, materials and labor needed for additional replication. Using the average Standard Error for each attribute from the previous feed ingredient study (Johnsen and Dupree, 1991), the range of values at error levels of 80, 85, 90 and 95 percent were generated.

Results of these tabulations, predicted score differences based on 2 to 50 replicates arranged in rows, were used in decision-making. As one example, the values depicting expected score variability if 5 replicates were conducted are included in Table 9. Providing a balance of panelist fatigue with potential ability to discriminate a difference, it was concluded that 5 replicates of descriptive flavor analysis would be performed per treatment with a power level β of 0.80 as a two-directional contrast. This method supports the test of the null hypothesis using a scale measurement that has meaning. In the previous feed ingredient study, statistical differences were found but were so small that it was uncertain what their impact was on overall catfish flavor. Therefore, the minimum detectable differences (MDD) for each attribute used to test if there was a difference from REF_CASE were: *Chickeny* 0.4, *Nutty* 0.4, *Fat Complex* 0.3, *Corn* 0.2, and *Sweet* 0.2.

Table 9. Results of power analysis calculations using previous channel catfish descriptive scores¹ to indicate a Minimum Detectable Difference (MDD) for each flavor attribute.

Calculated from scores at Time 0 days

	<i>Chickeny</i>	<i>Nutty</i>	<i>Fat Complex</i>	<i>Corn</i>	<i>Sweet</i>
Constant STD ERR ² used in calculations	0.25	0.27	0.17	0.14	0.14
Predicted MDD using 5 replications					
Power					
0.80	0.42	0.44	0.29	0.24	0.24
0.85	0.45	0.47	0.31	0.26	0.26
0.90	0.49	0.52	0.34	0.28	0.28
0.95	0.56	0.59	0.38	0.32	0.32

Calculated from scores at Time 300 days

	<i>Chickeny</i>	<i>Nutty</i>	<i>Fat Complex</i>	<i>Corn</i>	<i>Sweet</i>
Constant STD ERR ² used in calculations	0.36	0.23	0.14	0.16	0.10
Predicted MDD using 5 replications					
Power					
0.80	0.59	0.38	0.23	0.26	0.17
0.85	0.63	0.40	0.25	0.28	0.18
0.90	0.69	0.44	0.27	0.31	0.19
0.95	0.78	0.50	0.31	0.35	0.22

¹ from Johnsen and Dupree (1991)

² Standard Error

A second consideration in interpreting the DA panel results was that most panelists had an interaction of panelist with treatment. There was not a directional trend in these interactions, but they were significant for all five attributes ($p < 0.05$). Because detailed data on panelist performance had not been collected before these sessions, further analysis of panelists' responses was explored but not reported here (Powers, 1984). It had been assumed that the periodic maintenance panel sessions produced panelists with equivalent discriminatory skills. The multiple interactions showed that this was not true. The panel leader had observed trends of individual's difficulties with certain attributes, but complete data did not exist to warrant exclusion of data for an attribute by any individual panelist (*i.e.*, dropping outlier scores). Procedures to treat panel outliers were considered, but we concluded that the process was too severe for the amount of objective panelist performance data available (Powers, 1984; Bett *et al.*, 1993).

A stricter assessment was performed as a guide for choosing gas chromatography-olfactometry samples (described in Chapter 4). A trial of correcting the DA data was done as an exploration. If a trend showed, from tallying by attribute, that it was consistently rated opposite the panel, the score was changed to "." for missing data. This indicated the panelist could not discriminate that attribute consistently. Then the treatment ANOVA was run again. This exploratory operation helped compare the experimental treatments rigorously using only scores of those panelists who could discriminate. The DA treatment means after panelist-correction still did not show differences above the MDD criteria. So, while the panelist by

treatment interactions indicated that more attribute-practice and performance measurement should have been completed, there was no impact on the finding that differences were not observed. This procedure did identify feed treatment extremes that could be selected for odor analysis.

C. Sensory Evaluations

It was hypothesized that desirable flavors carried by the fat characteristics, as in marbling of beef or pork, would be perceivably higher in catfish with increasing time-on-diet (Lindsay, 1985; Manthey *et al.* 1988a; Huang *et al.*, 1994; Morris *et al.*, 1995). Mean intensities of *Fat Complex* were not significantly different and did not reflect the same increasing trend that was found for fillet fat. The genetically different groups in Study II were also not perceived as significantly different in *Fat Complex*. In Harvest 2, each genetic group demonstrated lower total fillet fat, but the sensory intensities stayed the same.

Use of BIFS for quantitative descriptive evaluation may have been one factor in reducing the variance of scores between assessors (Johnsen and Kelly, 1990; Wu *et al.*, 1996). Also, with the fillet fat content spread evenly in BIFS, the perceived intensity of other desirable flavors, *i.e.* *Chickeny* or *Sweet*, may have been higher than *Fat Complex* and maintained a constant overall flavor impact/perception. Reports by other investigators showed fatty characteristics dominating other fish flavors (Maligalig *et al.*, 1973; Manthey *et al.*, 1988a; Smith *et al.*, 1988). Serving protocols in these studies allowed the fatty edges of fillets to be distributed to panelists in an unbalanced fashion. In addition to the studies mentioned, Nettleton *et al.* (1990) and

Huang *et al.* (1994) reported fillet fat content with their sensory evaluations. Total fillet fat was comparable to the levels found here.

The intensity grand means for each flavor attribute over all replicates within a harvest for feed or genetic groups are summarized in Tables 10 and 11. In the Feed Effects study (Table 10), overall means for each attribute were not significantly different between groups ($p < 0.05$) and were not subjected to post-hoc testing. Mean scores by treatment subtracted from the same harvest REF_CASE mean were not greater than any MDD (Table 12). Table 11 shows that ANOVA calculations comparing Genetic Effect scores determined some means to be statistically different ($p < 0.05$). The differences were so small, however, that they did not meet the MDD criteria (Table 12).

Differences in Study II are also shown in Table 12, although it was not part of the null hypothesis to subtract the REF_CASE intensity means from genetics catfish flavor means. The procedure was done to illustrate the outcome that no genetic groups were perceived as different from the reference channel catfish. The only value greater than the MDD was *Chickeny* in BULL catfish in Harvest 1 (difference = 0.49 intensity units for BULL versus the MDD = 0.4 units for REF_CASE). This 0.09 scale difference would not be perceived by a trained panelist. A suggested limit of a Spectrum™ trained panelist's ability to discriminate is a minimum 0.5 on that scale (Gail V. Civile, personal communication). The difference does illustrate that in Harvest 1 the BULL group was most different from a selected reference catfish, such as REF_CASE. This difference also was evident in the triangle difference test.

Table 10. Grand least square mean intensities over 4 harvest sizes: catfish desirable flavor attributes in Study I, Feed Effects.

Flavor attributes	Experimental Ingredient ¹						P value	SEM ²
	REF_CASE	PRAC	CFML	MTBN	RICE	MOIL		
<i>Chickeny</i>	2.05	1.93	2.07	2.01	2.00	1.88	NS	0.040
<i>Nutty</i>	0.97	0.87	0.95	0.94	0.92	0.88	NS	0.028
<i>Fat Complex</i>	0.68	0.66	0.72	0.68	0.66	0.65	NS	0.019
<i>Corn</i>	0.46	0.44	0.46	0.39	0.39	0.40	NS	0.022
<i>Sweet</i>	1.01	0.91	0.98	0.98	0.96	0.95	NS	0.017

¹REF_CASE = Reference, Casein-base feed

PRAC = commercial catfish feed

CFML = 10% catfish meal feed

MTBN = 10% meat and bone meal feed

RICE = 10% rice bran feed

MOIL = 3% menhaden oil feed

²SEM = standard error of the mean

Table 11. Grand least square mean intensities over 2 harvests: catfish desirable flavor attributes in Study II, Genetic Effects.

Flavor attributes	Genetic Strain ¹							P value	SEM ²
	REF_CHAN_COMM	REF_CHAN_FARM	FAST	ALBI	BLUE	BULL	HYBR		
<i>Chickeny</i>	1.86 ^{bc}	2.12 ^a	2.10 ^{ab}	2.11 ^{ab}	2.08 ^{abc}	1.81 ^c	2.11 ^{ab}	0.01	0.091
<i>Nutty</i>	0.99 ^a	0.97 ^{ab}	0.97 ^{ab}	0.94 ^{ab}	0.85 ^b	0.89 ^{ab}	1.05 ^a	0.01	0.058
<i>Fat Complex</i>	0.70	0.75	0.73	0.69	0.76	0.72	0.75	NS	0.048
<i>Corn</i>	0.36 ^{bc}	0.57 ^a	0.49 ^{ab}	0.44 ^{abc}	0.43 ^{abc}	0.37 ^{bc}	0.49 ^{ab}	0.01	0.048
<i>Sweet</i>	0.98 ^{bc}	1.07 ^{abc}	1.13 ^a	1.02 ^{abc}	1.04 ^{bc}	0.96 ^c	1.15 ^{ab}	0.01	0.043

¹FAST = channel catfish, fast growing

ALBI = albino channel catfish

BLUE = blue catfish

BULL = black bullhead catfish

HYBR = hybrid channel x blue catfish

REF_CHAN_COMM = commercial channel catfish

REF_CHAN_FARM = farm-supplied channel catfish

²SEM = standard error of the mean

^{abc} Means within a row followed by same letters were not different at the P value

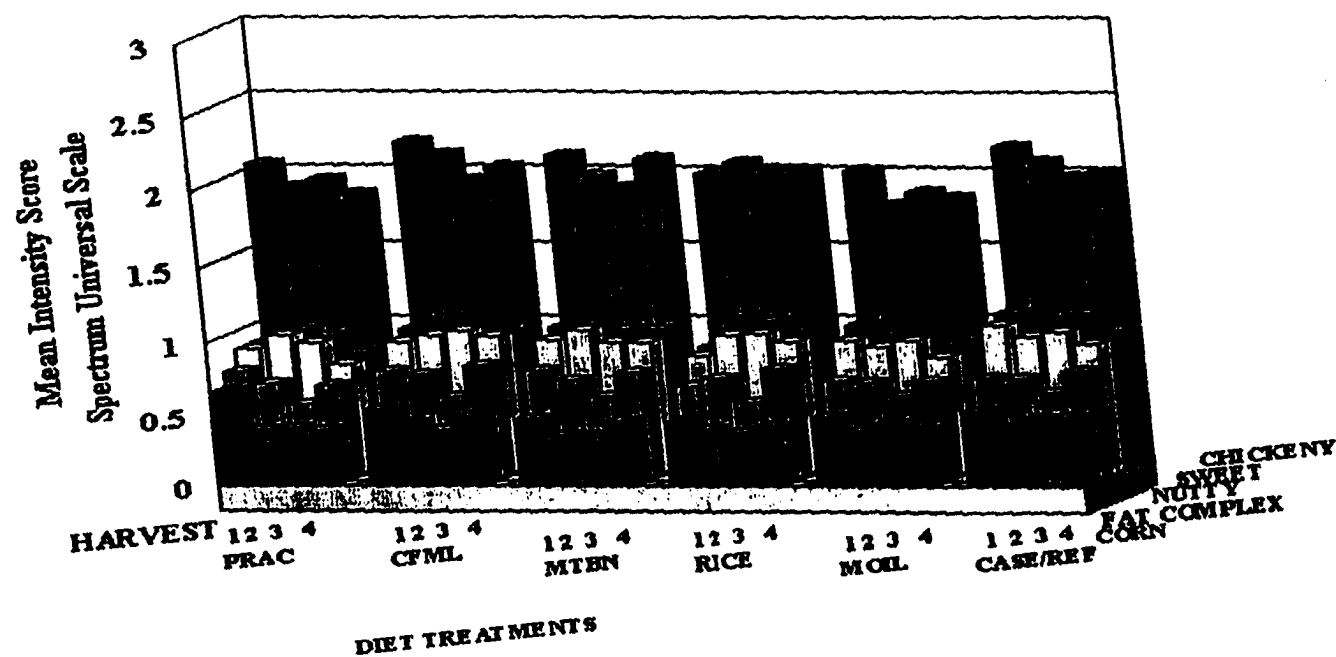
Table 12. Differences of mean scores from corresponding mean score for Reference, Casein (REF_CASE) treatment to analyze MDD criteria.

FEED FORMULATION						
	HARV	<i>Chickeny</i>	<i>Nutty</i>	<i>Fat Complex</i>	<i>Corn</i>	<i>Sweet</i>
PRAC	1	-0.13	0.16	0.06	-0.15	-0.19
	2	-0.19	0.00	-0.01	0.02	-0.07
	3	-0.04	-0.05	-0.07	-0.06	-0.11
	4	-0.16	-0.07	-0.08	-0.12	-0.12
CFML	1	0.02	0.02	0.07	-0.11	-0.11
	2	0.04	0.04	0.05	0.02	0.02
	3	-0.04	-0.06	-0.03	0.02	-0.10
	4	0.03	0.02	0.07	0.06	0.03
MTBN	1	-0.07	-0.05	0.05	-0.09	-0.09
	2	-0.10	-0.05	0.02	0.05	-0.02
	3	-0.09	-0.11	-0.03	-0.06	-0.07
	4	0.08	-0.02	0.01	0.03	0.04
RICE	1	-0.19	-0.07	-0.05	-0.21	-0.22
	2	-0.01	0.00	0.02	0.02	0.04
	3	0.04	-0.09	-0.07	0.00	-0.01
	4	0.02	-0.05	0.04	0.03	-0.00
MOIL	1	-0.16	-0.03	-0.02	-0.10	-0.08
	2	-0.29	-0.06	-0.02	-0.08	-0.10
	3	-0.11	-0.03	-0.03	-0.06	-0.06
	4	-0.17	-0.09	-0.07	-0.09	-0.07
GENETIC STRAIN						
	HARV	<i>Chickeny</i>	<i>Nutty</i>	<i>Fat Complex</i>	<i>Corn</i>	<i>Sweet</i>
FAST	1	-0.06	-0.01	0.09	0.02	0.18
	2	0.15	0.01	0.00	0.04	0.06
ALBI	1	0.04	0.01	0.06	-0.03	0.14
	2	0.07	-0.07	-0.05	-0.01	-0.12
BLUE	1	-0.01	-0.17	0.13	-0.03	0.10
	2	0.06	-0.07	0.02	-0.04	-0.04
BULL	1	-0.49	-0.19	0.05	-0.13	-0.05
	2	-0.00	0.03	0.02	-0.05	-0.05
HYBR	1	0.11	0.05	0.03	0.07	0.26
	2	0.01	0.10	0.10	-0.01	0.01
REF_CHAN_COMM	1	-0.19	0.02	0.02	-0.10	-0.03
REF_CHAN_FARM	2	0.07	-0.01	0.07	0.11	0.06

A flavor profile, or total impact, can be inferred by considering all five desirable attributes at once. The similar patterns of desirable flavor attribute scores due to Feed Effects or Genetic Effects are shown in Figures 3 and 4, respectively. Differences between these attribute means were not greater than the calculated MDD for each attribute (Table 12). The similarity of intensities considered together, as in eating, infers that perceived flavor impact for an individual would not be different between these types of catfish.

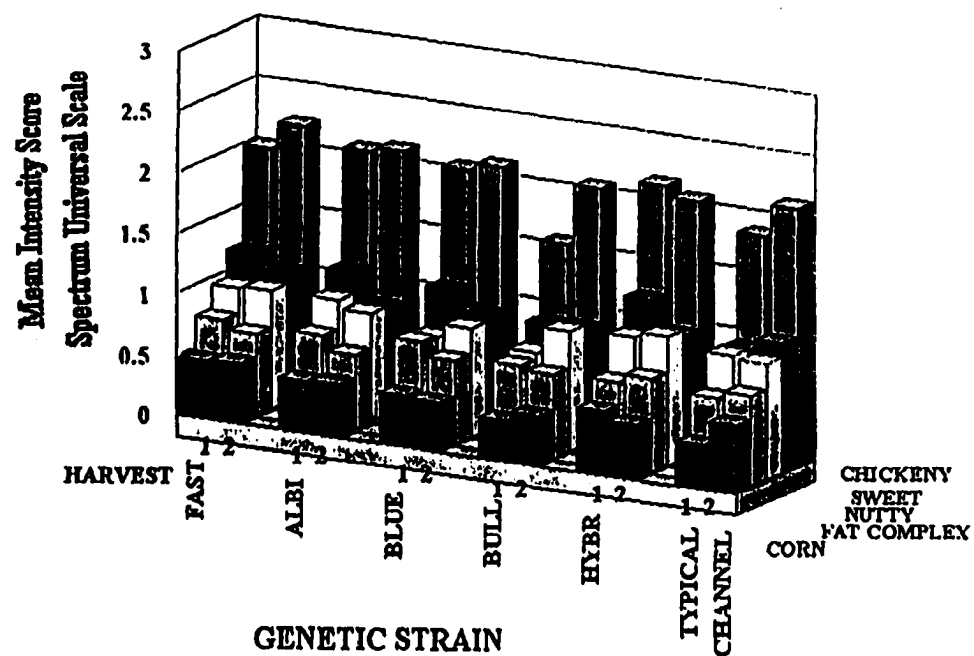
Triangle tests used as another sensory procedure to judge differences between treatments showed a few patterns of difference (Tables 13 and 14). In the Feed Effect study, the 18 panelists most often judged the MOIL feed catfish different from other diets ($p < 0.043$). The menhaden oil ingredient has distinct, marine-fish-like flavors. MOIL vs. RICE groups were judged as different in Harvests 2, 3 and 4. These were the most differences of any treatment pairing. Rice products generally have low-intensity flavors, and, compared to the MOIL product, a difference could be detected. Considering the perception of each of these ingredients by themselves, it appears that when the ingredients were converted to edible tissue by the catfish, the final flavors were in a similar, dichotomous range of intensity. This probably assisted panelists in judging the two treatments as different in all but the 165g fish.

During all time-on-diet (harvest sizes), each of the other feed treatments was found to be different from MOIL. Only PRAC was not found to be different from MOIL. For the most part, commercial catfish feed (such as PRAC) contains some portion of fish meal or fish by-product as an ingredient. Therefore, in the triangle test



PRAC = commercial catfish feed
 CFML = 10% catfish meal feed
 MTBN = 10% meat and bone meal feed
 RICE = 10% rice bran feed
 MOIL = 3% menhaden oil feed
 CASE/REF = Reference, Casein-base feed

Figure 3. Flavor intensity means over each harvest in Study I, Feed Effects.



FAST = channel catfish, fast growing
ALBI = albino channel catfish
BLUE = blue catfish
BULL = black bullhead catfish
HYBR = hybrid channel x blue catfish
TYPICAL CHANNEL 1 = commercial channel catfish
TYPICAL CHANNEL 2 = farm-supplied channel catfish

Figure 4. Flavor intensity means over each harvest in Study II, Genetic Effects.

Table 13. Results of sensory triangle difference tests in Study I, Feed Effects.

Feed Formulation	CASE	PRAC	CFML	MTBN	RICE
PRAC PRACTICAL Commercial catfish feed	★■				
CFML 10% CATFISH MEAL		■			
MTBN 10% MEAT AND BONE MEAL	•	•◆			
RICE 10% RICE BRAN		•■	•◆		
MOIL 3% MENHADEN OIL	■◆		★◆	•★	★■◆

Symbols denote significant difference within that harvest $p < 0.043$:

Harvest 1 (70 days on diet) = •

Harvest 2 (160 days on diet) = ★

Harvest 3 (210 days on diet) = ■

Harvest 4 (318 days on diet) = ◆

Table 14. Results of sensory triangle difference tests in Study II, Genetic Effects.

Strain of Catfish	REF ¹	FAST	ALBI	BLUE	BULL
FAST					
CHANNEL FAST GROWTH	•★				
ALBI					
CHANNEL ALBINO	•★				
BLUE					
BLUE CATFISH	•★				
BULL					
BLACK BULLHEAD ²	•	•	•	•	
HYBR					
HYBRID, Channel x Blue	•★				•
SLOW					
CHANNEL SLOW GROWTH	★			★	

¹Catfish samples were typical channel catfish from different sources during Harvest 1, REF_CHAN_COMM and 2, REF_CHAN_FARM

²Black Bullhead catfish evaluated in Harvest 1 only

³Slow-growing Channel catfish evaluated in Harvest 2 only

Symbols denote significant difference within that harvest $p < 0.043$:

Harvest 1 = •

Harvest 2 = ★

procedure (*i.e.* choosing one out of three samples that are different) it is likely that inclusion of fish ingredient flavors in PRAC made the two treatments not able to be judged as different.

Two trends were found in the genetic study triangle test comparisons (Table 14). First, the tests showed BULL catfish to be significantly different from all other strains. Even with only one presentation (Harvest 1), we expected that panelists could choose the BULL sample in all comparisons. The DA methods had harvest duplication, but the five attributes were not found significantly different. We believe the balance of flavor attributes in BULL catfish was dominated by other descriptive attributes not calculated in this study (unpublished data). They were observations reported as “Other” attributes, like *chemical-plastic*, *beefy*, *sour* and *marine seafood-like*, each time they were presented. The overall impact of BULL flavor attributes was distinguishable from all other genetic strains. However, black bullhead catfish are not candidates for large-scale aquaculture production because of their low dress-out (Table 4) and aggressive behavior (Robinson, 1989). Their characteristics were investigated here to collect information on common catfish in the southern United States growing region.

The second trend established with triangle testing was that all genetic groups could be differentiated from the typical reference catfish, coded REF_CHAN. In Harvest 1, even though the REF_CHAN_COMM was flavor checked after purchase by the industrial method (Johnsen, 1995), levels of geosmin and/or MIB could be detected by the trained panel. The catfish fillets had not been judged as off-flavor by

the flavor checking method. The fish fillets were used as the warm-up fish and in one experimental treatment. Panelists reviewed these perceptions in their discussion period and then continued with their normal DA protocols. Then when BIFS from the same preparation group, REF_CHAN_COMM, were presented in the triangle tests, they were judged as different ($p < 0.043$). The influence of geosmin or MIB (intensity 1-2 in the warm-up fish on the Spectrum™ scale, unpublished data) most likely rendered them distinguishable.

In Harvest 2, an effort was made to recreate a reference fish sample with similar characteristics. REF_CHAN_FARM catfish were supplied by the aquaculture facility of Delta Branch Experimental Station, Leland, Mississippi. The history of the fish in this farm facility was known, and the fish had been rated by an experienced industry flavor checker as a level 3 intensity “bluegreen” (0, absent to 5, highly intense scale). Again, the REF_CHAN_FARM samples were determined to be different from all other genetic groups (Table 14). This was likely due to the presence of geosmin or MIB. The industry flavor checking scale and Spectrum™ scale have not been correlated to determine equivalent benchmarks on each, but it appeared that the two environmental flavors made trained panelists able to perceive the differences.

Summary

The sensory methods designed to cross-verify differences in catfish flavor produced inconsistent results. Use of Spectrum™ DA scale evaluations did not establish significant flavor differences by treatment or harvest. Triangle tests, however, showed a number of differences when sample pairs were considered side by

side. MOIL, PRAC, REF_CHAN_COMM, REF_CHAN_FARM and BULL were distinguishable. The discrepancy in results leads us to conclude that differences exist. They appear to be small but noticeable. Since the basis of the substituted feed formulations was a semi-purified casein diet with indoor growing conditions, we speculate that the low intensity differences found by these judges would not be perceived in fish grown under more typical circumstances. Fifty percent of the REF_CASE treatments were found different from the PRAC commercial feed in which fish, soy and other by-products are likely to have been mixed. These ingredients, with their inherent flavors, plus uptake of environmental substances from surrounding pond water would render any low intensity feed-substitution differences to be judged the same by end-users. Further, it does not seem to be an advantage for producers to grow catfish to exceptionally large sizes to provide flavor development related to fillet fat.

CHAPTER 4

THE NATURE OF AROMAS IN OIL FROM COOKED FARM-RAISED CATFISH

Introduction

Flavor characteristics of farm-raised fish are an important factor in their acceptance (Johnsen, 1991). Representative flavor perceptions described in channel catfish by a multi-product panel are white meat-like, nutty/buttery and vegetative notes (Chambers and Robel, 1993). These low-to-moderate amplitude primary flavors were also described in industry research and may potentially be dominated by environmental compounds (Johnsen *et al.*, 1987; Johnsen and Kelly, 1990).

Flavor evaluations by industry staff can be costly in manhour and financial terms. Trends in quality assurance toward replacement of human sensory evaluations with instrumentation have been considered. At this time, instruments cannot duplicate human ability to perceive and communicate overall food impact complexities. In the seafood industry, several traditional quality control procedures have been conducted by human perception (Johnsen, 1991). The human nose performs complex operations and combines with mental sensory references to interpret the stimuli perceived. Some fish products require odor evaluation for freshness. However, in the aquaculture industry, fish are evaluated to prevent undesirable off-flavor compounds from reaching commercial distribution (Johnsen, 1995). Economic losses and harvest delays due to environmental off-flavors are important issues for farmers.

To improve on human limitations, such as fatigue or nasal blockage, instrumental techniques generally focus on better detection of aroma compounds at low levels (Pollien *et al.*, 1997). Aroma separation techniques in farm-raised catfish

have been prioritized with off-flavor detection (Johnsen and Lloyd, 1992; Johnsen, 1995). Mills *et al.* (1993) published a survey of aroma compounds in typical retail channel catfish. Understanding instrumental analyses for innate, preferred flavors would benefit industry screening of fish for desired characteristics. More instrumental data on desirable flavors is needed to interpret overall aroma characteristics and the balance of off-flavors.

Instrumental food analyses to examine flavor matrices employ several approaches. At this time, instruments cannot mimic the human ability to perceive overall food impact complexities. Fractions of the food yield subsets of the total flavor constituents. For example, peptides, carbohydrates and volatile compounds make up separate fractions that require different analytical conditions. Solubility, heat requirements, volatility and ease of separation from the food product are some factors that determine which fraction is produced. A significant factor is assuring that the fraction is representative of the original food. Symposia of recent research findings continue to update the considerations needed to generate a high quality flavor sample (Ho and Manley, 1993; Maarse and van der Heij, 1994).

Recently, more fishery products have been investigated during development of flavor analysis instrumentation (Przybylski *et al.*, 1989; Josephson *et al.*, 1991a, 1991b; Medina *et al.*, 1997). One promising approach is gas chromatography-olfactometry (GCO). The GCO method categories of aroma extract dilution analysis

(AEDA) (Ullrich and Grosch, 1987), Charm analysis (Acree and Barnard, 1994), and time-intensity related, Osme (da Silva *et al.*, 1994) aim to measure the impact of food aromas. The combination of gas chromatography (GC) column separation and real-time odor evaluation continue to show relationships back to the flavor of the whole food. The techniques are evolving, and several authors have hypothesized ways to improve GCO methods. These include understanding the meaning of the food matrix before extracting (Pollien *et al.*, 1997), improving extraction methods (Abbott *et al.*, 1993a; Taylor and Larick, 1995), understanding the limitations of panelists (Abbott *et al.*, 1993b; van Ruth *et al.*, 1994), and further examining aspects of human perception (Taylor and Linforth, 1994). Combining objective instrumental detection and human perception/naming of volatile chemical compounds creates synergy in determining the odor impact of compounds (da Silva *et al.*, 1994).

One food category that has been moderately well studied by GCO is fishery products. Studies from this university laboratory have reported lists of volatile compounds that make up freshwater and marine species (Tanchotikul and Hsieh, 1989; Matiella and Hsieh, 1990; Cadwallader *et al.*, 1994). Fish oils have been examined by Karahadian and Lindsay (1989), and volatile compounds in whitefish (Josephson *et al.*, 1983) and smoked fish (Sakakibara *et al.*, 1990) have been recounted. Josephson and his coworkers (1991a, b) have determined several aspects of fresh and ocean salmon aromatic compounds.

A preliminary GCO investigation of farm-raised catfish compared raw and cooked channel catfish volatile compounds (Mills *et al.*, 1993). Minor differences

were seen in raw and cooked flavors from catfish fat. These few differences were confirmed using flavor-by-mouth evaluations with an experienced panel. Mills *et al.* (1993) found 41 compounds in both cooked and uncooked fish, mostly aldehydes and alkyl benzenes. However, the channel catfish were from a commercial retailer, and little was known about the environment from which the fillets were produced.

The main objective of this study was to examine a range of farm-raised catfish oil extracts for volatile odor compounds and determine if marker compounds eluted in a specific pattern. A second objective was to determine if a statistical relationship existed to predict by-mouth flavor intensities (determined in Chapter 3) from GCO data.

Materials and Methods

A. Training Sniffer Panelists and Conditions

Organization of a GCO panel began when a group of 12 subjects participated in screening exercises to determine their ability to discriminate and name odors. The exercises included evaluation of a few flavor and taste samples and participation in one session of GCO effluent sniffing. Exercises were planned to cover a broad range of skills, to expose the subjects to familiar and unfamiliar activities, and to observe their performance (adapted from Meilgaard *et al.*, 1991). From the 12 subjects, three GCO panelists were chosen based on performance and availability. The author also participated as a panelist. Overall, the panel consisted of four women ranging in age from 22 to 58 years.

Subjects completed 15 training sessions prior to GCO data collection. The initial sessions were group meetings to experience and discuss a large number of reference products (Appendix D). These products had characteristic odors, for example *rubber cement*, and all panelists experienced the same products then communicated to the others what terminology they would use. Shared terms were from individual's mental frame of reference (*i.e.* sensory memory). This served to instruct the other panelists in the group. Consensus of terms was not required and therefore was a modified free-choice profiling technique (Quarmby and Ratkowsky, 1988).

Two of the 15 training sessions were spent practicing with the intensity references for flavor-by-mouth assessment used in allied sensory panel evaluations (Chapter 2, Table 1). Sessions included both by-mouth and by-nose techniques. Practice with reference products (Meilgaard *et al.*, 1991) built a psychological context of increasing intensity. The panel agreed to use the following category scale, adapted from the Spectrum TM intensity scale: *weak aroma* = intensities 1, 2, or 3 / *moderate aroma* = intensities 4, 5, 6, or 7 / *intense aroma* = intensities 8, 9, 10 or higher. The term "*nothing*" was agreed to express a zero value, or absence of any odor. The exercises served to harmonize the group, broaden the panelists' experiences, as well as gain understanding of and practice with the aroma perception tasks.

During seven training sessions, individual panelists practiced the activity of sniffing samples at the GCO sniffer port. The new panelists needed to develop a context of the GCO operations and gain practice in the rapid responses needed with an

unknown sample's sequential odors. To reduce fatigue, periods of sniffing were 5 min-on/5 min-off. The solvent eluted during time period 0-5 min, so this portion was not evaluated. The training method was an adaptation of the basic training scheme for panelists using a flavor-by-mouth descriptive analysis technique. The author adapted the philosophies of training from Meilgaard *et al.* (1991), Civille and Lawless (1986) and personal experience as a sensory panel leader.

B. Extraction of Oil from Cooked Catfish

All glassware was washed in detergent, rinsed with tap water, rinsed five times with deionized, distilled water, air-dried, and baked at 220°C for 2 hr.

A modified method of Mills *et al.* (1993) was used for all procedures.

Volatiles from a range of farm-raised catfish conditions (Table 15) were analyzed to survey farm-raised catfish. Samples in two categories of extract storage, Frozen or Fresh (Section D, below), were prepared for analysis by GCO.

To acquire material for extraction, composite shredded catfish muscle was prepared at the same time as sensory panel samples (Appendix A) (Johnsen and Kelly, 1990). Shredded material from each treatment group to be analyzed by GCO was packaged in 2.9 mil nylon/saran/polyethylene vacuum bags, flushed with nitrogen, and vacuum-sealed. Packages of shredded material were held at -18°C and used within one year. Thirty different treatments were extracted twice with aliquots of these extracts further analyzed.

Table 15. Designated sample codes for gas chromatography-olfactometry analysis of farm-raised catfish.

CATEGORY		FEED FORMULATION	STUDY	NO. OF EXTRACTS	
STORAGE CONDITIONS	ORIGINAL TREATMENT			HARVEST	
FROZEN	REF_CASE	Reference, Casein- base feed	FEED	1	2
				2	2
				3	2
				4	1
FROZEN	CFML	10% Catfish Meal in casein-base	FEED	1	2
				3	1
FROZEN	MOIL	3% Menhaden Oil in casein-base	FEED	2	2
				3	1
FROZEN	FAST ¹	35% protein commercial fingerling diet	GENETIC	2	2
FROZEN	HYBR ²	35% protein commercial fingerling diet	GENETIC	1	2
FRESH	TYPC_FEED	32-34% protein lab typical formulation	NEW	1	2
FRESH	REST_CHOI	28% protein commercial diet	NEW	not	1 ³
				applicable	1 ³
FRESH	COTTON	28% protein commercial diet	NEW	1	1

¹ channel catfish "fast-growing", *Ictalurus punctatus* Kansas x Kansas

² hybrid catfish, channel *Ictalurus punctatus* Red River x blue *Ictalurus furcatus* Blue

³ extracts were not repeated measures from one shredded composite

Table 15 shows the categories of samples analyzed by GCO. The samples categorized as Frozen were extracted within one year of the 1991-1992 harvest dates, then stored in freezers (Section D). Selected substituted feeds were chosen for GCO analysis from Study I, Feed Effects. The method of selection was based on a determination of desirable flavor attribute means after non-discriminating panelists were replaced as missing data. From this rigorous treatment of the data the two flavor intensity extremes were taken, CFML and MOIL. Samples were further chosen for analysis within these groups if their extracts had not evaporated over the storage period. The Fresh category in Table 15 designates recently extracted channel catfish samples. These samples were selected to survey typical farm-raised catfish from university production farms, TYPC_FEED, or individually-quick-frozen institutional-packed fillets, REST_CHOI. One sample of channel catfish fed a semi-purified diet, COTTON, was also analyzed by the GCO method. In this way, the effect of the storage could be estimated.

To extract oil, samples were cooked in a manner similar to descriptive analysis panel samples. The frozen blocks of composite shredded fish in vacuum-sealed packages were approximately 20 cm x 15 cm x 2.5 cm thick. These shredded fish blocks were cut while frozen into cubes approximately 2.5 cm x 2.5 cm x 2.5 cm to provide more surface area for cooking. Cubes of frozen shredded catfish were placed in 15 cm x 20 cm polyethylene/polypropylene coated pouches (Dazey Corporation, Industrial, Kansas) and heat-sealed (Appendix A). Target weight of fish for each

extraction was $850\text{g} \pm 150\text{g}$. Cubed, sealed cooking pouches were either used immediately or held at -18°C and used within 24 hours.

Three pouches of fish were placed in each of two 15-quart pots of rapidly boiling water and covered with lids. Total boiling time was 6 min, after the water returned to a boil. When the water returned to rapid boiling, the cooking pouches were periodically arranged so that the fish cubes were evenly exposed to the boiling water. One minute of cooking time was added to the procedure for sensory panel preparation to accomplish heat penetration of the fish cubes.

The cooked fish and juice were centrifuged in 250-ml polycarbonate bottles at $14,700 \times g$ for 30 min at 25°C . The temperature was critical to prevent formation of a congealed emulsion. After centrifugation, about 2/3 of the watery bottom layer was drawn off using an aspirator with a glass Pasteur pipette. The remaining water/fat mixture was poured into a polyethylene screw-cap centrifuge tube. The liquids were allowed to drain from the polycarbonate bottle for 10 sec for repeatability. A second centrifugal procedure of $4000 \times g$ for 6 min at 25°C separated the water and oil layers. As much of the catfish oil layer as possible was transferred to a 1000-ml glass 3-neck flask using a glass Pasteur pipette. The oil obtained was weighed and recorded.

C. Extraction of Volatiles from Fish Oil

A fixed amount of $3 \mu\text{L}$ of Internal Standard (I.S.) per gram of oil was added to the catfish oil obtained from each treatment. The I.S. contained $10 \mu\text{g}/\mu\text{L}$ each of 2,3-dichloropyrazine and benzothiophene (thionaphthalene) in n-hexane. Headspace

volatiles were collected from extracts by purging with a stream of nitrogen (20 ml/min for 6 hr) through each extract onto a Tenax GC 60/80 mesh trap. The glass 3-neck flask was suspended in a water bath at 65 to 70°C throughout the procedure.

The duration of purging was reduced from 16 hr (Mills *et al.*, 1993) to 6 hr because there was concern that the 16-hr method of purging volatiles exposed the catfish oil to elevated temperatures too long and that heat artifacts could be generated. The technique also required overnight purging that was unmanageable for 74 extraction samples. The 6-hr period was adopted after a Tenax trap breakthrough study was completed. Patterns of volatiles that broke through the Tenax trap to a second trap at 2, 4, 6 and 8 hours were determined. The 6-hr trapping duration was established because a small number of compounds transferred to the second trap.

After 6 hrs, the volatiles were eluted from the trap with 10 ml redistilled ethyl ether. The traps were corked and held at -12 °C up to two days, thawed, then eluted. The solvent was evaporated to < 500 µL with a stream of nitrogen directed onto the solvent surface and then transferred to a 1 ml glass, crimp-top sample vial.

D. Extract Storage

Those sample vials in the Frozen category (Table 15) were held at -12 to -70 °C for a wide range of times of up to 6 years. Exceptions to freezer temperatures were as follows: freezer failure for 2 days during a hurricane, August, 1993; air freight transport on dry ice to Palmerston North, New Zealand, January, 1995; return air freight transport on dry ice from New Zealand to New Orleans, Louisiana, November,

1995; freezer failure for 7-10 days for unknown reason, May, 1996. Fresh category sample vials were held at -10 to -18 °C for up to 4 months.

E. Gas Chromatography-Olfactometry of Volatiles

The gas chromatograph (Hewlett Packard model 5890A with model 5895A data system, Avondale, Pennsylvania) was equipped with a flame ionization detector (FID), a DB-5 capillary column (30 m x 0.53 mm, film thickness 1 µm; J & W Scientific, Deerfield, Illinois), and an effluent splitter. Flow rates of hydrogen and air were 1.5 ml/min and 2 ml/min, respectively. For the sensory evaluation of the GC effluents, the chromatograph was modified with a sniff port consisting of a 5 cm pyrex funnel. Effluents were mixed with humidified air. The GC had an injector temperature of 220°C, a detector temperature of 200°C, and a helium flow rate of 3 ml/min. The GC was temperature programmed from 35°C to 196°C at 3.5 °C per min. Sample injection volume of 10 µL was kept constant after redistilled ether was added to reconstitute each extract to 100 µL +/- 20 µL.

Verbal descriptions of odor stimuli during GCO were recorded along a timeline for that extract, with columns for each panelist's evaluation. The timeline data included term(s) for the odor, beginning and ending time, intensity, and other comments about the changing stimulus as it occurred. The retention times (RTs) of descriptive terms were later matched to the RTs on the gas chromatogram.

Data analyses were mean values of starting and ending times per stimuli and tallies of intensity scores. If at least one panelist scored *moderate* intensity for an

odor, all panelists' scores at that RT were tallied. The odor terms were counted. Descriptive panel data and odor intensity scores were associated by the canonical correlation (CANCORR) procedure of SAS (SAS Inc., 1985).

GC-mass spectrometry measurement of volatiles was performed on a Hewlett Packard GC-MS (model 5971 with Windows Release C Data Analysis System, Avondale, Pennsylvania). A DB5-MS column (20 m x 0.18 mm, film thickness 0.18 μm ; J & W Scientific, Deerfield, Illinois) was installed. The GC-MS had an injector temperature of 200°C, a detector temperature of 196°C and was heat programmed from 35°C to 196°C at 3.5°C per min with a 2-min hold at the beginning and 80-min hold at the end of the run. Sample injection volumes were 1, 2 or 3 μL from the same reconstituted extracts (100 μL +/- 20 μL) to achieve a response above the detection threshold but not overloading to the column.

Results and Discussion

A. GCO Panelist Performance

Panelist training assisted each individual in becoming comfortable with the tasks but did not appear to reduce the number and variety of descriptive terms used (Appendix D). With GCO experience, panelists indicated that an odor had been perceived previously and became more consistent in giving the stimuli a similar descriptive term. Some perceived odors could only be given an overall category name, like *chemical* or *non-food-like*. While other authors have not discussed category terms (Ullrich and Grosch, 1987; Pollien *et al.*, 1997), this has been typical of the results of

other food-sniffing teams (unpublished data, Owen E. Mills, John R. Vercellotti and Carol A. Kelly). Using category terminology at least leads the researcher to possible sources that may have produced the stimuli.

Using a human transcriber to record start time and duration of odor stimuli helped reduce panelist confusion in performing several tasks at once, which was a criticism of intensity methods reported by Pollien *et al.* (1997). When the procedure includes a transcriber, two individuals are dedicated to the task and it is very time consuming. In this study, 74 runs were required to evaluate the catfish extracts by the 5 min-on/5 min-off procedure. This equates to 74 runs for 2 individuals for 1 hour each, or 148 hours, not including data interpretation.

An added complication in GCO was the amount of variation between panelists' terminology for compounds (as described in the Olfactometric Analyses section below). Abbott *et al.* (1993b) and da Silva *et al.* (1993) have discussed data analysis approaches needed for adequate interpretation. Approximating the variability of response terminology here, this panel was in agreement with those findings. The source of variation was usually that the concentration level of a compound was below an individual panelist's detection threshold, which rendered that panelist unable to perceive and give a response (Meilgaard *et al.*, 1991; Pollien *et al.*, 1997). Other panelists might perceive that particular odor, but miss another one to which the first panelist responded. The high frequency of this phenomena is a limitation in exactly matching the results of one GC run to another (Abbott *et al.*, 1993b). An effort was made to inject the same concentrations of extract from the vial. It was beyond the

scope of this investigation to quantify the peak area of each compound; instead the time and character of odor responses were key.

Another source of variation was intuitively opposite; if the compound was too concentrated from a normally injected sample, then the panelist's descriptive term may have varied. Panelist terminology variation decreased as each panelist gained more experience. Each panelist was more likely to use a similar descriptive term for the same stimuli with practice. This improvement in repeatability occurred as a panelist's context became more developed, which was in agreement with Civile and Lawless (1986).

The ability to perceive, interpret and verbalize a stimulus in rapidly changing clusters of compounds is challenging to an individual. Retention times 19 through 26 minutes exhibited this in farm-raised catfish extracts. The final terminology recorded could be different, depending on the absence or presence of a transcriber who interacts. Some terms may be missed or unrefined without a transcriber because the panelists could only give a vague term at the time of the stimuli. With methods in which the panelists do not interact with a transcriber, panel members may not be able to think quickly enough or may not have enough clear experiences in their sensory memory from which to draw the correct terminology. Another consequence is that panelists often recall a term later and want to express it. Panelists may be more effective using an audio recording device in conjunction with recording the odor duration on a piece of paper, as suggested by Pollien *et al.* (1997). If panelists wish to refine the term given to a perception, they can speak into the audio recording device.

Without another person present, the individual would not be distracted into another context. The panelist's response would be totally independent of other individuals. This improvement of the sniffing port technique, suggested by Pollien *et al.* (1997), would reduce total transcriber time because the recorded tape would only have data when someone spoke. In addition, there would be a reduction in the long ranges of the response "*no odor perceived*" for the transcriber. Also, as stated before, there would be less psychological bias by the panel leader/transcriber.

The limitations found here in odor evaluation methods could be improved by adopting the toggle-type-button technique for duration, as well as the voice tape recorders cited by Pollien *et al.* (1997). If intensity data is desired, it could also be expressed into the tape recorder. It may be less time-consuming to transcribe an audio tape that had been recorded by voice activation than to engage a second person in transcribing during an entire session.

Because these were semi-quantitative screening evaluations, it was possible for the panel leader/transcriber to verbally explore with the panelists the most appropriate descriptive term for the stimuli. If the study were entirely quantitative, it would not be prudent to have a transcriber who interacted with the panelists. The practice could lead to artifacts of terminology due to bias in trying to give the most "correct answer." Semi-trained panelists have often been observed as trying to express "the right answer," perhaps to please the panel coordinator or "help" the final outcome (Meilgaard *et al.*, 1991). The transcriber could also lead the panelists in a direction that really was not their context. Practice and descriptive training would also benefit

data collection by making the panelists more reliant on external references and less on individual sensory memory.

B. GCO Analyses of Catfish Oil

1. Gas Chromatography Traces

Several patterns of volatile catfish compounds eluted on GC traces (Figure 5). The GC traces are shown within one figure to compare the patterns. Figures 8 through 17 in Appendix E detail the pattern of each treatment using a single, representative GC trace.

The observed patterns were similar within a category class linked by treatment-plus-harvest, *e.g.* REF_CASE Harvest 1, with some variation in amplitude. These similarities of pattern repeated within a category but less between categories. Since 148 GCO runs were necessary to accommodate the 5-min sniffing periods, the similarity of GC traces within a treatment is notable. This suggests that the extraction method was highly reliable in separating compounds from each composite fish sample. The differences in patterns between categories suggest that one type of GC trace would characterize one composite shredded fish material. This would argue for developing GC techniques based on catfish for which growth factors are known. The established pattern(s), particular to the objectives of a business or laboratory, could be used to screen test catfish against some established criteria.

Distinctive clusters of peaks produced patterns within a treatment GC trace. Examples are the stacked wide peak at Minute 8 to 10 in CFML extracts (Figures 5b, 11) and several moderate size peaks in two clusters during Minute 15 to 22 in both

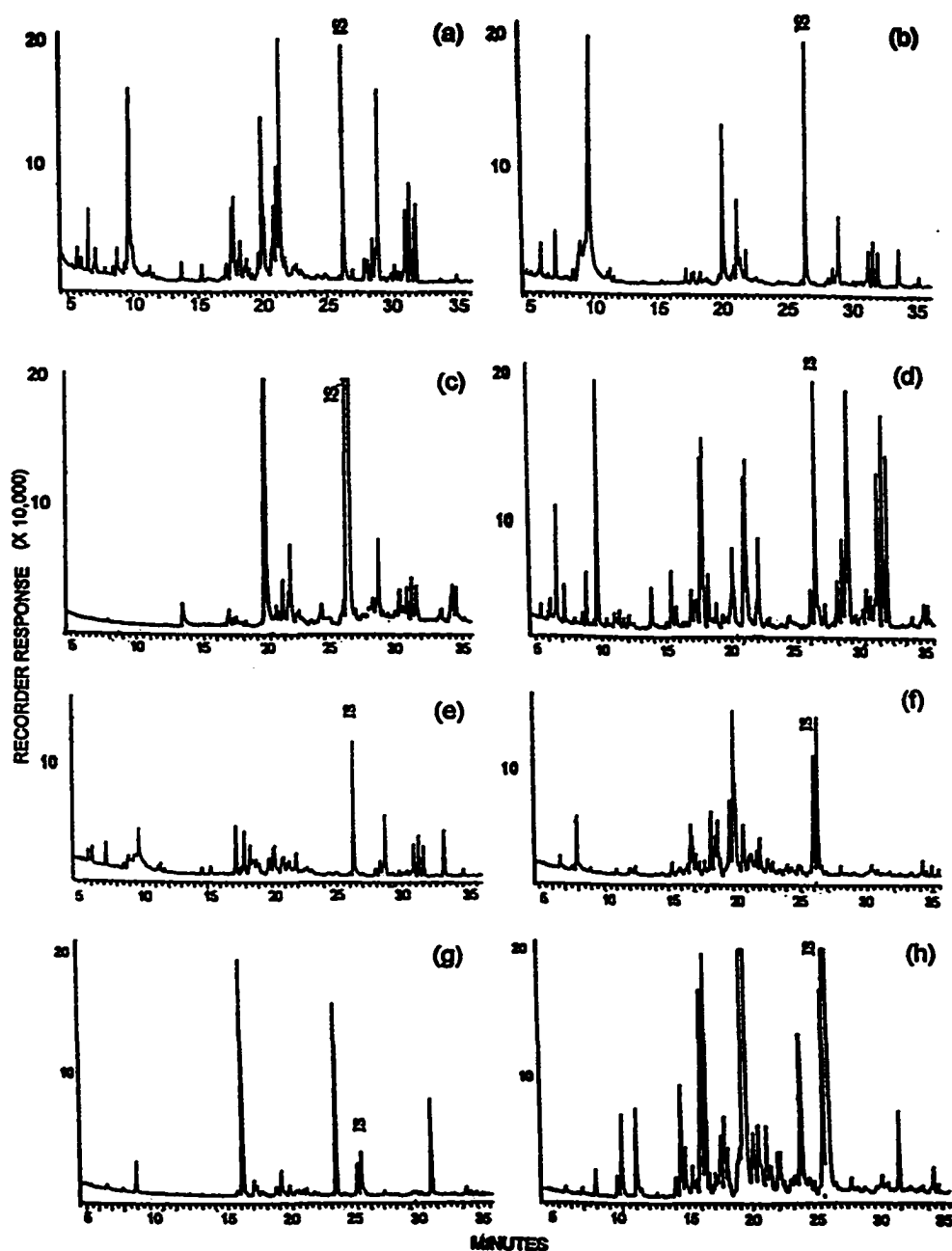


Figure 5. Illustrative chromatograms of volatile compounds isolated from catfish oil from eight treatments: (a) REF_CASEIN, (b) 10% CATFISH MEAL, (c) 3% MENHADEN OIL, (d) FAST-GROWING CHANNEL, (e) HYBRID CHANNEL x BLUE, (f) TYPICAL_FEED CHANNEL, (g) COTTONSEED_MEAL CHANNEL, (h) RESTAURANT'S_CHOICE.

REF_CASE, Harvest 1, 3, and 4 (Figures 5a, 8, 10) and REST_CHOI (Figures 5h, 16) were determined. Conversely, it can be observed that the lack of peaks at Minute 5 to 19 in MOIL (Figures 5c, 12) also creates a repeated pattern.

Whether the patterns of GC flavor volatiles in fish raised under typical farm conditions would be as repetitive is unclear. Flavor volatile patterns have been used to differentiate quality groupings in canned tuna fish association to flavor acceptability (Przybylski *et al.*, 1991). Mills *et al.* (1993) found repeated compounds in raw and cooked catfish using shredded fish from commercial distribution. Few studies have reported the large number of samples evaluated here. The time investment needed for purge and trap techniques suggests that each laboratory would be able to develop a GC pattern database of catfish flavors only for their particular interest.

The two Internal Standards did not emit odors. The RT for benzothiophene was calculated from standard curves of direct injections and verified by mass spectrometry. The compound was identified in 73% of the GCMS extracts. Similar verification for other compounds is not reported here because benzothiophene was the only substance above the instrument's detection threshold, thus allowing it to be identified. Since the benzothiophene was still present within samples whose peak amplitudes were in normal range by GC, this confirmed that the Frozen storage samples had not deteriorated beyond practical use. Presence of this spiked compound that was trapped on Tenax, stored and recovered in liquid diethyl ether after 6 years inferred that other compounds driven off from the catfish oil were also present. The RT for 2,3-dichloropyrazine was determined from GC standard curve data only.

2. Olfactometric Analyses

Odors were analyzed in two different ways. In Table 16, descriptive terms were used to describe stimuli as they eluted. Twenty perceived odors met the *moderate* intensity criteria at RT 5 through 31 minutes, with odors beyond 31 minutes more irregular. The descriptive words have been compiled in Table 16 as communicative terms that were perceived by panelists. The terms are not in any particular order within one cell of the table. Terms are expressed by category names if that did not misrepresent the actual perception. The mean RT range over 74 GC runs was carried over from Table 17. Overall occurrence means are derived from frequency statistics by category in Appendix F.

An effort was made to keep the sample concentration uniform so that each panelist would be evaluating the same amount of material. This method has advantages for recording the duration and intensity of odorants, thus describing the balance of aroma perceptions that represent the original food extract (Reineccius, 1993; da Silva *et al.*, 1994; Pollien *et al.*, 1997).

At each RT, the odor terms seem to cluster and describe a perceived character of that stimuli. Identity of suggested compounds in these effluents (Table 16) is based on RT comparisons of this data with mean values of the authentic compounds. Comparing the incidence and character of odors in the catfish examined here, 35% of the compounds are common to the published catfish term list (Mills *et al.*, 1993). We

Table 16. Recurrent odors perceived from sniffing port evaluation of farm-raised catfish treatments.

Odor Label	Descriptive Terms of Odors Perceived in Extracts	Retention Time Range ¹	Authentic Compound in n-hexane solvent	Mean Ret. Time ²	Articles in which Compounds Previously Reported ³
FIV	sweet, fruit, candy,	5:20-5:41			
SIX	burned leaves or rubber, vinyl	6:57-7:23			
SEV	green grassy	7:32-8:21	hexanal	7:52	A,B,C,D,E,F,G,H,I,J
EIG	citrus, floral	8:20-8:55			
TEN	fruity, swimming-pool-like, floral, oil paint, fishy, solvent, glue	10:04-10:31			
ELE	sweet chemical, fruity, glue, latex, overheated coffee	11:22-11:37	heptanal	13:18	A,B,C,D,E,F,G,H,J
TWEL	fishy (50%panel) or toasted crust of honey-wheat bread	12:15-12:58	dimethyl pyrazine	12:26	A,C,D,G,K
THIRT	roasted nuts or decaying vegetation/over-cooked green beans or grassy/cucumber	13:33-14:16			L terms = caramel, toasted cereal, body odor
FIFT	sweet floral or rotten cabbage, metal-like, decaying vegetation	14:57-15:30			
SIXT	mushroom, overcooked mushroom, burned vegetation, metal, green decay	16:06-16:52	1-octen-3-ol	16:48	A,B,C,D,E,H,K
SEVT	lemony, floral, cucumber, sweet, fruity, grassy	17:13-18:03	2-pentyl furan	17:20	A,C,G,J
NINT	burning or sweet chemical, vinyl, leaves of houseplants, fish after storage, dry cleaning store	19:39-20:20	octanal	18:38	A,C,D,E,G,I

(table continued)

Odor Label	Descriptive Terms	Retention Time Range ¹	Authentic Compound in n-hexane solvent	Mean Ret. Time ²	Articles in which previously reported ³
First I.S.	Internal Standard		2,3-dichloropyrazine	19:20	
TWEN	solvent, musty, grassy, floral, hairspray, alcohol, green decay	20:34-21:29			L terms = 2-methyl-2-borene
TTWO	soapy, fruity, radish, chlorine, dusty, sweet chemical, body odor, cucumber, mildew	21:50-22:42	d-limonene	22:06	A,F,G
TTHR	cherry , sweet but not fruity, cucumber/grassy	22:20-23:39			L terms = benzaldehyde
TFIV	hot vinyl, chemical , cucumber floral, linseed oil , mown hay, toasted wheat	24:57-25:46			L terms = toasted protein
Second I.S.	Internal Standard		benzothiophene	26:15	
TSIX	cucumber, grass	25:36-26:41	nonanal	26:01	A,B,C,D,E,F,G,I,J
TSEV	rotting green grass, chemical, solvent, acetone, turpentine, sawdust, painty, celery, dust, mushroom-dirt, rubber cement, burning grass	27:09-28:33	decanal	27:23	E,J
TNIN	rubber cement, chemical, grassy	28:59-29:59			L terms = painty/ Aldehyde
THIR	mothball, burning plastic or dust, rubber cement, vinyl, latex paint	30:02-32:01	2-methyl naphthalene	30:45	A

(table continued)

Odor Label	Descriptive Terms	Retention Time Range ¹	Authentic Compound in n-hexane solvent	Mean Ret. Time ²	Articles in which previously reported ³
	canned vegetable, burned vegetable or toast vegetable oil or cooking oil, or cottonseed-oil processing plant, musty, baked potato skin, linseed oil, latex or oil paint, solvent, plastic, rubber	> 31:00 min continuing to the end			

¹From Table 17

²n=6

³Articles citing other food products that contain these volatile compounds:

A = farm-raised catfish (Mills *et al.*, 1993)

B = fresh salmon (Josephson *et al.*, 1991a)

C = dried fish products (Sakakibara *et al.*, 1990)

D = baked or baked, canned salmon (Josephson *et al.*, 1991b)

E = whitefish (Josephson *et al.*, 1983)

F = blue crab (Matiella and Hsieh, 1990)

G = crayfish waste (Tanchotikul and Hsieh, 1989)

H = alligator (Cadwallader *et al.*, 1994)

I = French beans (van Ruth *et al.*, 1995)

J = chicken extracted by supercritical CO₂ (Taylor and Larick, 1995)

K = wild rice (Withycombe *et al.*, 1978)

L = farm-raised catfish, unpublished terminology data (Owen E. Mills, 1993)

Table 17. Mean starting and ending times of odor stimuli from farm-raised catfish extracts.

ODOR LABEL	OCCURRENCE ¹ (%)	MEAN (MINUTE)	STD DEV	START TIME (MINUTE)	
				MINIMUM	MAXIMUM
FIV	51.4	start 5:119	0.092	5:00	5:53
		end 5:413	0.389	5:12	7:51
SIX	32.4	6:565	0.176	6:20	7:34
		7:231	0.156	7:01	8:03
SEV	70.3	7:321	0.113	7:15	8:08
		8:213	0.331	7:29	10:01
EIG	35.1	8:199	0.203	7:59	9:06
		8:545	0.243	8:14	9:50
TEN	62.2	10:036	0.164	9:37	11:10
		10:308	0.381	9:50	12:26
ELE	29.7	11:222	0.225	10:30	11:49
		11:373	0.265	10:44	12:24
TWEL	68.9	12:146	0.136	11:52	13:05
		12:578	0.246	12:13	14:13
THIRT	64.9	13:332	0.215	13:09	14:45
		14:159	0.279	13:31	15:15
FIFT	67.6	14:568	0.189	14:22	15:45
		15:300	0.262	14:58	16:38
SIXT	95.9	16:062	0.107	15:50	16:38
		16:519	0.233	16:29	17:51
SEVT	87.8	17:131	0.219	16:31	19:19
		18:029	0.359	17:25	19:50
NINT	67.6	19:386	0.276	18:32	20:30
		20:199	0.359	19:11	22:44
TWEN	73.0	20:342	0.283	20:00	22:44
		21:290	0.370	20:45	25:00
TTWO	74.3	21:497	0.189	21:15	22:21
		22:421	0.451	21:54	25:00
TTHR	62.2	22:199	0.195	21:54	23:50
		23:386	0.481	22:40	25:24
TFIV	83.8	24:567	0.147	24:17	25:31
		25:455	0.612	25:05	30:00
Internal Std ²		26:090		benzothiophene	
TSIX	70.3	25:358	0.219	25:18	26:54
		26:406	0.494	25:46	30:00
TSEV	74.3	27:094	0.275	26:13	28:30
		28:333	0.439	27:20	30:00
TNIN	56.8	28:592	0.262	28:02	29:52
		29:589	0.348	29:30	32:23
THIR	89.2	30:022	0.391	28:34	33:00
		32:006	0.985	30:15	35:15

¹Total Extracts = 74

²Did not emit odor. Peak seen in 90% of extracts and verified by mass spectrometry in 73% of extracts.

conclude that more than 35% would match with published GC trace aromagrams if the term-identification lists are considered. The trend of association is speculated from odor terms. Additional recorded data was available to the author from unpublished results archived at USDA_ARS_SRRC (personal communication, Owen E. Mills). These notes further support the hypothesis that these samples and the raw and cooked catfish had many overlapping constituents. Thirty compounds were listed in Mills' report, compared to 20 that met the *moderate* level criteria in these results. No new compounds that had not been reported in catfish or other food products were found in these samples.

The compounds that recurred in both published studies were hexanal (FIV), heptanal (ELE), dimethyl pyrazine (TWEL), 1-octen-3-ol (SIXT), 2-pentyl furan (SEVT), octanal (NINT), and 2-methyl naphthalene (THIR). Previous work determined that these compounds were present in both raw and cooked catfish, with the exception of dimethyl pyrazine in raw only and 2-methyl naphthalene in cooked only (Mills *et al.*, 1993). Examples of the unconfirmed overlapping odors (coded THIRT, TWEN, TTWO, TFIV and TNIN) are detailed in the column "Previously Reported" in Table 16.

Note that the last column in the table lists other reports of compounds found in this study. Hexanal (SEV), heptanal (ELE), 1-octen-3-ol (SIXT) and nonanal (TSIX) have been found in a range of fishery (Josephson *et al.*, 1983, 1991a, 1991b; Sakakibara *et al.*, 1990), chicken (Taylor and Larick, 1995) and one bean (van Ruth *et al.*, 1995) product. The floral/cucumber note of 2-pentyl furan (SEVT) was less often

reported, specifically in dried fish, crayfish and chicken. Mills *et al.* (1993) reported it in both raw and cooked catfish. A less common compound, d-limonene (TTWO), occurred in 74.3% of the samples in this study and was identified by Mills and coworkers but did not generate an odorous response in their work. This compound was reported in crab and crayfish, but it is unclear how much this relates to a fish-type impact because of its intermittent perception in the GCO effluent.

Another descriptive compound of interest, dimethyl pyrazine, has been found in heated fishery products, but not in fresh whitefish. Two descriptors were given for this effluent, *i.e. toasted crust of honey-wheat bread* versus *fishy*, which seem incompatible, but two panelists always called it *fishy*, and another consistently called it *toasted crust*. Most likely, this descriptor is perceived differently at different concentrations over the range of GC runs. However, because it is not in all finfish products, it might be a factor in the perceived quality of *nutty* when catfish is evaluated by-mouth.

The sniffing technique produced several descriptive terms per odorant compound, except for *green grassy* (SEV), *mushroom* (SIXT), and *mothball/chemical* (THIR) odors. This is consistent with the results of a free-choice profiling technique (Quarmby and Ratkowsky, 1988). All representative terms have been listed to demonstrate the variety and range of perceptions elicited when samples are evaluated by more than one judge. If a multivariate modeling technique of collapsing the terms was preferred, the procedure would only be valid if there were an even larger number of data points than the 74 GCO runs evaluated here. The free-choice profiling

technique is nonetheless valuable. Catfish volatile GC traces were found to vary in pattern between treatment, but the odor occurrence and character were more similar (Table 16 and Appendix F). This is in agreement with results found in French bean products by van Ruth *et al.* (1995).

The most frequently used descriptors were in the vegetative categories, *e.g.* *grassy*, *cucumber*, *floral*, *fruity* or *mushroom*. This agrees with the fish characteristics found in both catfish (Mills *et al.*, 1993) and salmon (Josephson *et al.*, 1991b). These vegetative odors were often accompanied by burnt, toasted or heated odors and variations of decaying vegetation (Figure 5), also found by Mills in both the raw and cooked catfish. At several retention times, odors were regularly perceived that could be characterized as *vinyl-like*, *rubber cement/solvent/plastic*, or *paint-like*. Mills *et al.* (1993) showed these *chemical-like* descriptors on aromagrams of both cooked and raw catfish samples but did not include them in the list of identified compounds. Here, in catfish samples that had been extracted within a month of harvest (*i.e.* FAST and HYBR) and also in samples stored long-term, these non-foodlike odors were included (Figure 6). The exact origin of these odors is not readily apparent because they can be confused with odors in the *fishy-like* category. It is unclear if they are by-products of lipid oxidation also found in fish (Josephson and Lindsay, 1986).

Flavor volatiles described by sniffer port panelists in this study were only from the oil fraction, to which heat had been applied for 6 hr. The results show an abundance of solvent-plastic-type odors that may have been generated by this heat treatment. Compounds like dimethyl pyrazine and naphthalene were also reported in

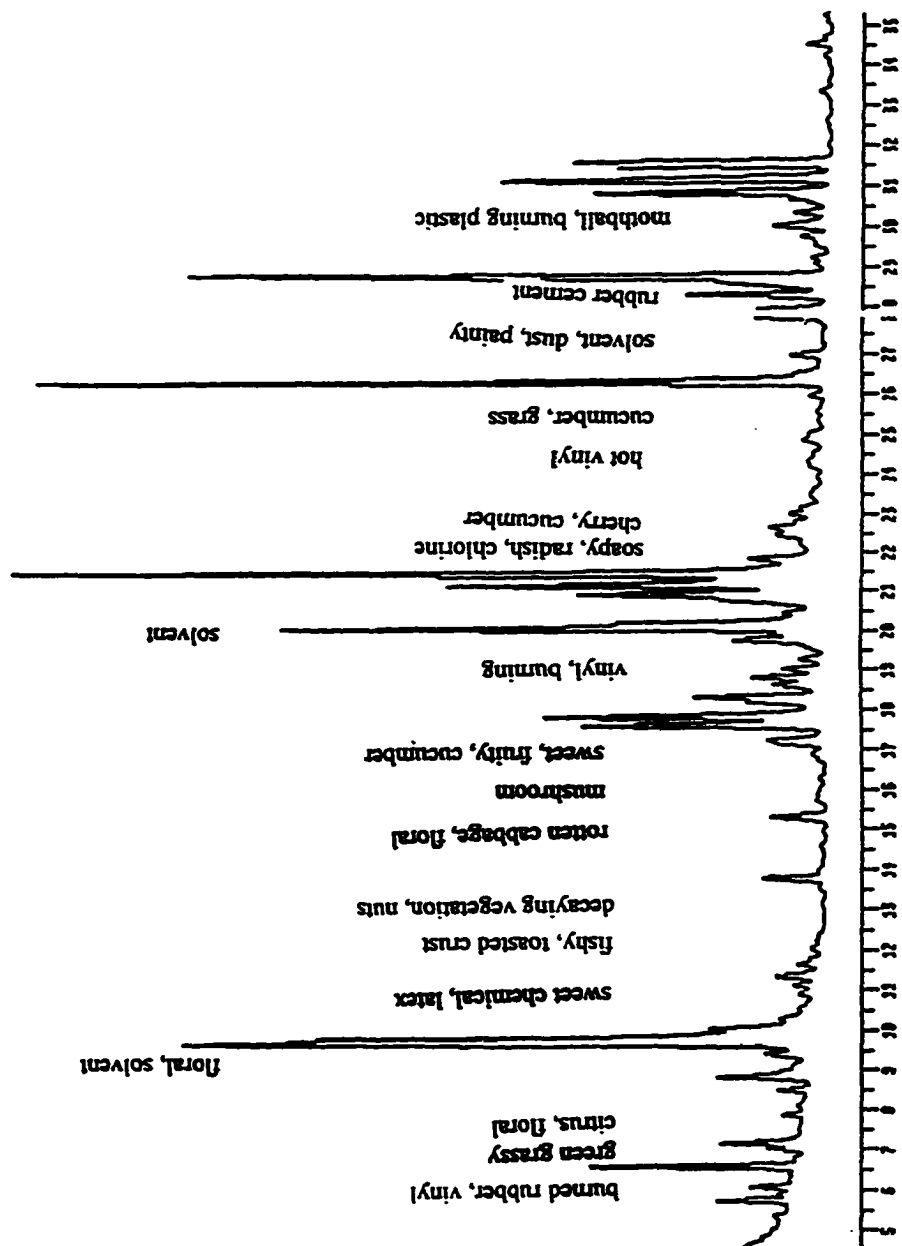


Figure 6. Farm-raised catfish odor compounds from catfish oil of a representative treatment in Feed Effects, Study I.

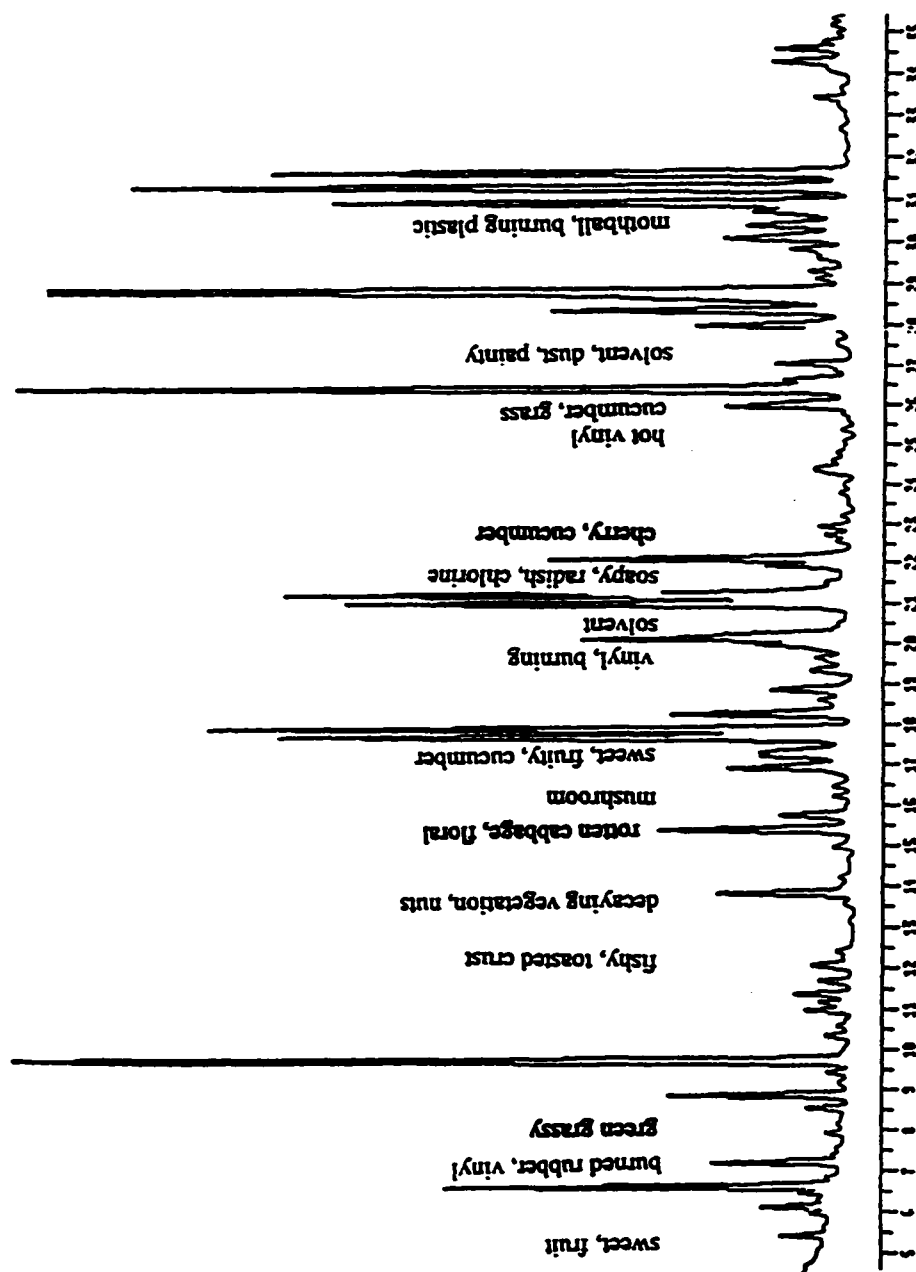


Figure 7. Farm-raised catfish odor compounds from catfish oil of a representative treatment in Genetic Effects, Study II.

smoked or dried fish (Sakakibara *et al.*, 1990) and in canned salmon with further dry heat treatment Josephson *et al.* (1991b). The former is speculated to be a Maillard reaction product and, interestingly, was not found in whitefish (Josephson *et al.*, 1991), crab (Matiella and Hsieh, 1990) or crayfish products (Tanchotikul and Hsieh, 1989). Unknown compound, FIV (*sweet, fruit*), was described more frequently in genetic study extracts, with SIX, EIG and ELE at such low frequencies that they are not included on the representative aromagram (Figure 6). These four effluents did not seem to have odors that corresponded with those found by Mills *et al.* (1993).

By general observation, it seems that most odor compounds from catfish were in a vegetative category. These odors were in a range from *green grassy* and *cucumber* to *decaying vegetation* and *dry, mown hay*. While the data did not show a trend to explain this by feed or genetics, it is commonly perceived that vegetable-like flavors make up a noticeable part of the balance of catfish flavor in farm-raised catfish. The term “vegetative” was one of the key terms used by a trained panel evaluating freshwater fish (Chambers and Robel, 1993). When one chews a piece of catfish, the major impact is chickeny and sometimes buttery, but a noticeable part of the balance tastes like green vegetables, *e.g.* green beans or English peas. It is not surprising, then, that a large number of vegetative compounds were perceivable in the catfish GC effluents.

A consistent perception of nutty did not manifest itself, and the odors perceived with terms like *cooking oil* and *the outside of a cottonseed oil mill processing plant* were not in a pattern that could infer a causative compound. Decanal

is sometimes termed *old-oil* in character and may have been the source. Another explanation is that each ether extract had been exposed to conditions that would favor autooxidation and that some degradation products were perceived. Ullrich and Grosch (1987) did an early study on the breakdown products of linoleic acid. They found that hexanal and heptanal were common breakdown products, and they were found here. Hexanal is in a large range of foodstuffs, including fresh salmon (Josephson *et al.*, 1991b), chicken (Taylor and Larick, 1995), and beans (van Ruth *et al.*, 1995). There are other pathways of formation not related to lipid oxidation. Few other aldehydes were perceived in these samples. By contrast, many hydrocarbons were found, and the carbonyl side chains may have broken down during storage. However, the interesting outcome was that all the extracts still had flavor even if they had to be reconstituted. This suggests that ethyl ether was a good medium in which to freeze the samples. The trade-off was its ease of evaporation if not securely capped.

C. Correlation of Descriptive Flavor and Odor Analyses

Canonical correlation is best used with small data sets that do not lend themselves to multivariate statistics (MacFie and Hedderley, 1993). It is a linear comparison of one data-set's ability to predict another. The by-mouth evaluations did not find differences between treatments for the five flavor attributes. Odor intensity rather than frequency were used as the variable for the odor factor. It was presumed that no weight would be lost because each occurrence had a corresponding intensity score. Similar to factor analysis, the canonical correlation procedure collapses the data, but it is appropriate for smaller data sets. Canonical correlation then reports

probabilities of prediction for one set of variables from the other. The most conservative interpretation uses the last stepwise values after the redundancy analysis built into the procedure.

One interesting preliminary result was that before factoring *Sweet* flavor correlated to a high degree opposite all other desirable flavors, *i.e.* its coefficient was negative when the others were positive and vice versa. None of the five attributes were significantly different due to feed or genetic effects, so no explanation for this result can be given.

After checking the five collapsed factors for redundancy, the data showed the amount of variance explained by Sensory or Odor factors. Only 68.2 % of the data was explained when factors 1, 2 and 3 for Sensory were accumulated and through Factor Five, which explained 67.5% of the Odor data. These are not indicative of a strong correlation. Alternatively, strong correlation would show this level of cumulative variance explained by Factor 1 or 2. For individual attributes, high rates of explained variance did not occur until Factor 3. The intensity of odor NINT (octanal) was predicted at 90.0%, odor TFIV (unknown) at 88.1%, and odor TSIX (nonanal) at 86.4% by the third factor. In the Sensory data, *Corn* flavor was predicted at 81.3%, *Sweet* flavor at 70.1%, and *Chickeny* flavor at 67.1% by the third factor.

Conclusions

GCO techniques employed to survey farm-raised catfish odor characteristics generated 20 odors that recurred at a moderate to high incidence. The occurrences are

notable when considering the limitations of the methodology including separation of compounds, human inconsistencies, and portion of the food product.

The 20 substances discussed in this report have been found in other vegetative and animal products. While odors perceived as *green grassy*, *mushroom* and *mothballs* occurred reliably, perception of their intensity was not strongly predicted. Their occurrence in numerous other food products and variability in these data rendered them unable to be named marker compounds. If the objective of this study was to investigate all flavor compounds in farm-raised catfish oil, then one may conclude that use of repetitive catfish gas chromatography patterns within a linked category class may be an effective laboratory practice.

Since 148 GCO runs were completed to accommodate the 5-min sniffing periods, the similarity of GC traces within a category class is notable. It suggests that the extraction method was highly reliable in separating a class of compounds from each composite fish sample. The differences in patterns between categories suggest that one type of GC trace would characterize one catfish strain plus the influences of its feed and environment. This would argue for developing GC techniques based on patterns of catfish for which growth factors are known. The established pattern(s), particular to the objectives of a business or laboratory, could be used to screen test catfish against some established criteria.

CHAPTER 5 SUMMARY AND CONCLUSIONS

Flavor issues are highly valued in the farm-raised catfish industry. Frequently, environmental conditions dominate the flow of business by affecting the fish with muddy/musty off-flavors from microorganisms in the ponds. Such episodes are costly in terms of dollars and labor. As the industry seeks more accurate predictive tools for these episodes, individuals in each business sector continue striving to provide a high quality product.

While control and elimination of the off-flavor problem is a priority, the overall flavor impact of catfish products warrants attention to maximize its desirable qualities. Most research reporting flavor evaluations of farm-raised catfish has used untrained, screening-type panels. Over the last decade, focused work made possible by commitment to fundamental catfish flavor knowledge did establish a trained panel at the USDA-ARS-SRRC, New Orleans, Louisiana. The descriptive flavor analysis panel was enlisted to elucidate solutions to the off-flavor problem and analyze aspects of desirable flavors in catfish.

This investigation employed the panel as one tool in assessing low magnitudes of flavor differences in catfish that were grouped by semi-purified diets or genetic strain. The rigor of sensory methods was increased by cross verification with two tests, *i.e.* descriptive analyses and triangle difference tests. This approach was effective because the desirable flavor intensity technique did not show differences between any treatments. Desirable flavor intensities also did not increase as fillet fat

increased, as was expected. The triangle tests concluded that, with samples side by side, some differences could be perceived.

In conjunction with sensory evaluation of desirable flavors, samples evaluated by the trained taste panel were extracted for analysis of flavor volatiles by gas chromatography-olfactometry. The purpose of this study was to determine if the presence of volatile compound patterns derived from pond, spawning or diet factor were useful as predictors of desirable catfish flavor. The scope of catfish products in this survey was diet, one hybrid channel x blue, and length of storage samples. Qualitative visual differences in volatile GC patterns were distinctly seen between categories of treatments. Few pattern differences occurred within categories. But the resultant odor stimuli character and pattern eluted from the extracts were quite similar. No new odors from food were found, and the ubiquitous nature of the flavor compounds found in other vegetative and animal products did not lead to marker compounds for desirable catfish flavors. The procedures are time-consuming and only provide information on volatile compounds that can be generated from the catfish oil fraction. The techniques may be useful for screening or estimation purposes. The association of these odor data with trained panel flavor data by canonical correlation did not find strong predictive relationships.

The inconsistency of by-mouth perceptions indicates that small but noticeable differences exist. Fifty percent of the Reference-Casein feed treatments was found different from the practical commercial feed. In commercial feed, materials like fishmeal, soy and other bulk byproducts are usually mixed. If ingredients like these with their inherent flavors were combined with volatile compounds from pond

conditions, then the overall flavor impact would be similar to catfish that had had any common feed ingredient substituted at its normal level for nutrition. The flavor perception as an entire eating experience would be complex. A range of flavors would be acceptable and would become known as characteristic or typical, but not unbalanced. This argues for least-cost formulation rather than any special formulation that produces more distinguishable desirable flavor characteristics at grow-out.

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APPENDIX A

PREPARATION OF BLENDED INDIVIDUAL FISH SAMPLES (BIFS)

The preparation steps outlined below aim to reduce sensory between-sample variation (Johnsen and Kelly, 1990).

A. Preparing BIFS:

1. Skin catfish with a mechanical skinner (Jacard model A35-P membrane skinner, Orchard Park, New York)
2. Trim excess skin or bone; fillet; record total fillet weight of the two fillets from one fish carcass.
3. Compile all fillets in a tared, chilled bowl; record that as total fillet weight.
4. Cut fillets into 2-3 inch pieces and place in food processor receptacle, approximately half full, to shred pieces. Shred 4 seconds exactly.
5. Compile all shredded material into tared, chilled bowl by scraping the meat out of the food processor with a stainless steel spoon. Continue with pieces of fish until all shredding is completed. Mix the composite catfish sample thoroughly with an institutional size stainless steel spoon.
6. Using a stainless steel portioning-scoop, place 1 scoop (10g) into a 7.5 cm x 10 cm polyethylene/polypropylene boiling pouch (modified by making 4 smaller pouches from one pouch (dimensions 15 cm x 20 cm, Dazey Corporation, Industrial, Kansas)).
7. Freeze at -18°C for 4 weeks or less.

B. Preparing GC-extraction samples:

1. Place the shredded material (above) as 250g +/- 5g portions into nylon/saran/polyethylene vacuum pouches (2 mil thickness). Flush with nitrogen while drawing a vacuum. (Note: polyethylene/polypropylene coated pouch may be used if the anticipated storage time before analysis is 3 weeks or less.)
2. Heat-seal the bags.
3. Freeze for minimum 24 hrs at -18°C before preparation for gas chromatography.

APPENDIX B SELECTED PROGRAMMING LANGUAGE TO SHOW USE OF A CALCULATED STANDARD-ERROR TO PREDICT STATISTICAL POWER LEVEL

Example 1:

```

proc sort data=TWO; by PANEL TRT GROUP HARV

proc summary MAXDEC=2 data=TWO;
VAR CHY NTY FCX CRN SWT;
by PANEL TRT GROUP HARVEST;

output OUT=CASE_X MEAN=CASE_CHY CASE_NTY C
run;
data CASE_X; set CASE_X;
if TRT='G6' then delete;
run;
proc print by PANEL TRT GROUP HARVEST;
run;

data CASE_X(drop=TRT GROUP _TYPE_ _FREQ_);
run;
proc sort data=CASE_X; by PANEL TRT GROUP
run;
proc print data=CASE_X;
TITLE1 'MEAN SCORE OVER PANELIST OF CASEI
run;

data EXPDIET; set TWO;
if TRT='G6' then delete;
run;
proc sort data=EXPDIET; by PANEL TRT GROUP
run;
proc print data=EXPDIET;
TITLE1 'RAW SCORES OF PANELISTS OF EXPERI
DESIRABLE ATTRIBUTES';
run;

data DIFFS; merge EXPDIETX CASE_X;
by PANEL;

DIFFCHY = CHY - CASE_CHY;
DIFFNTY = NTY - CASE_NTY;
DIFFFCX = FCX - CASE_FCX;
DIFFCRN = CRN - CASE_CRN;
DIFFSWT = SWT - CASE_SWT;
run;

proc sort data=DIFFS; by PREFIX PANEL TRT
run;

```

```

proc print data = DIFFS;
  TITLE 'INTENSITY DIFFERENCES OF CASEIN AT
        var PREFIX PANEL TRT GROUP REP CODE H
        CASE_CHY CASE_NTY CASE_FCX CASE_C
        CHY_NTY FCX_CRN SWT
        DIFFCHY DIFFNTY DIFFFCX DIFFCRN D
run;

```

Example 2:

```

DATA d.se; SET d.se (KEEP=c_gsm c_mib c_dvg c_grv c_chy c_nty c_fcx
                    c_crn c_cbd c_pty c_fsh c_swf c_sty c_btr c_ppy);
RUN;
TITLE3 'STANDARD ERROR ESTIMATES FOR DIFFERENCE BETWEEN TWO MEANS';
PROC PRINT DATA=d.se; RUN;

/*****/

/* DETERMINE MINIMUM DETECTABLE DIFFERENCE BETWEEN MEANS FOR
   N=2 TO N=50 REPS WITH POWER=90% AND 95% */

TITLE1 'COMPUTING MINIMUM DETECTABLE DIFFERENCE BETWEEN MEANS WHEN
POWER IS 90%';
TITLE2 'SAMPLE SIZES CONSIDERED ARE 2 TO 50 - LEVEL OF SIGNIFICANCE IS .05';

DATA d.power90; SET d.msepower;
FILE PRINT;
PUT ' ';
PUT 'N D_GSM D_MIB D_DVG D_GRV D_CHY D_NTY D_FCX D_CRN D_CBD
D_PTY D_FSH D_SWT D_STY D_BTR D_PPY';

PUT ' ';
DO N=2 TO 50;
  D_GSM= ( SQRT((2*FINALGSM)/N) )*( TINV(.975,N-1) + TINV(.90,N-1) );
  D_MIB= ( SQRT((2*FINALMIB)/N) )*( TINV(.975,N-1) + TINV(.90,N-1) );
  D_DVG= ( SQRT((2*FINALDVG)/N) )*( TINV(.975,N-1) + TINV(.90,N-1) );
  D_GRV= ( SQRT((2*FINALGRV)/N) )*( TINV(.975,N-1) + TINV(.90,N-1) );
  D_CHY= ( SQRT((2*FINALCHY)/N) )*( TINV(.975,N-1) + TINV(.90,N-1) );
  D_NTY= ( SQRT((2*FINALNTY)/N) )*( TINV(.975,N-1) + TINV(.90,N-1) );
  D_FCX= ( SQRT((2*FINALFCX)/N) )*( TINV(.975,N-1) + TINV(.90,N-1) );
  D_CRN= ( SQRT((2*FINALCRN)/N) )*( TINV(.975,N-1) + TINV(.90,N-1) );
  D_CBD= ( SQRT((2*FINALCBD)/N) )*( TINV(.975,N-1) + TINV(.90,N-1) );
  D_PTY= ( SQRT((2*FINALPTY)/N) )*( TINV(.975,N-1) + TINV(.90,N-1) );
  D_FSH= ( SQRT((2*FINALFSH)/N) )*( TINV(.975,N-1) + TINV(.90,N-1) );
  D_SWT= ( SQRT((2*FINALSWT)/N) )*( TINV(.975,N-1) + TINV(.90,N-1) );
  D_STY= ( SQRT((2*FINALSTY)/N) )*( TINV(.975,N-1) + TINV(.90,N-1) );
  D_BTR= ( SQRT((2*FINALBTR)/N) )*( TINV(.975,N-1) + TINV(.90,N-1) );
  D_PPY= ( SQRT((2*FINALPPY)/N) )*( TINV(.975,N-1) + TINV(.90,N-1) );
  PUT @1 N @5 D_GSM 5.3 @12 D_MIB 5.3 @19 D_DVG 5.3 @26
    D_GRV 5.3 @33 D_CHY 5.3 @40 D_NTY 5.3 @47 D_FCX
    5.3 @54 D_CRN 5.3 @61 D_CBD 5.3 @68 D_PTY 5.3
    @75 D_FSH 5.3 @82 D_SWT 5.3 @89 D_STY 5.3
    @96 D_BTR 5.3 @103 D_PPY 5.3;
END;
RUN;

```

APPENDIX C

SELECTED PROGRAMMING LANGUAGE TO SHOW UNIVARIATE ANALYSES OF DESCRIPTIVE SENSORY DATA

Example 1: SAS procedure used in split plot ANOVA of Study I, Feed Effects.

```
PROC MEANS NOPRINT; BY REP TRT HARVEST;
VAR CHY NTY FCX CRN SWT;
OUTPUT OUT=MNS MEAN= MN_CHY MN_NTY MN_FCX
      MN_CRN MN_SWT;
RUN;
PROC GLM DATA=MNS;
CLASSES REP TRT HARVEST;
MODEL MN_CHY MN_NTY MN_FCX MN_CRN MN_SWT =
REP TRT REP*TRT HARVEST TRT*HARVEST;
TEST H=TRT E=REP*TRT;
MEANS REP TRT;
MEANS REP TRT/TUKEY LINES E=REP*TRT;
MEANS HARVEST TRT*HARVEST/TUKEY LINES;

LSMEANS TRT/STDERR PDIFF E=REP*TRT;
LSMEANS HARVEST TRT*HARVEST/STDERR PDIFF;
RUN;
```

Example 2: SAS procedure used in ANOVA of Study II, Genetic Effects.

```
PROC MEANS NOPRINT; BY REP G_STRAIN HARVEST COMB;
var CHY NTY FCX CRN SWT;
OUTPUT OUT=MNS MEAN=MN_CHY MN_NTY MN_FCX
      MN_CRN MN_SWT;
RUN;
data four; set two;
proc sort data=four; by G_STRAIN HARV REP PANEL;
RUN;

proc glm data=MNS;
classes REP G_STRAIN HARVEST;
model MN_CHY MN_NTY MN_FCX MN_CRN MN_SWT =
      REP G_STRAIN HARVEST REP*G_STRAIN;
test H=G_STRAIN
E=REP*G_STRAIN;
means REP G_STRAIN;
means REP G_STRAIN/TUKEY LINES E=REP*G_STRAIN;
lsmeans G_STRAIN/STDERR PDIFF E=REP*G_STRAIN;
RUN;
```

APPENDIX D
GENERAL GROUPINGS OF VOLATILE DESCRIPTIVE TERMS
USED BY GCO PANELISTS FOR FARM-RAISED CATFISH
AND METHODS TRAINING.

Green Grassy Geosmin or "Dry Musty" Green Slime Rotten Cabbage Moldy or "Wet Musty" Mildew Brackish-green water Sweaty socks, fresh Sweaty socks, old and "ripe" Green or Musty category	Vinyl or General Plastic Chlorine-like Odor of Natural Gas Mothball-like Isopropyl Alcohol or Ethanol or Methanol or Hydrogen Peroxide Sweet Chemical Formaldehyde Acetone Rubber Cement or Benzene Toluene or "Dry Cleaning Store" Tetrahydrofuran or "2 day-old Trout" Turpentine-like Ether-like Medicinal Benzoyl Peroxide Vinegar Chemical category	Vanilla Mint Anise or Licorice Cotton Candy or "Candy Floss" Honey Bubble gum Chewing Gum, mint-type Yeasty (like rising bread) Chocolate Menthol Celery Cucumber Garlic Potato Food-like category
Vegetable Oil odor Latex Paint Oil-Based Paint Odor outside a Cottonseed Oil Processing Plant Linseed Oil Castor Oil Cooking Oil Buttery Painty category	Boiled/Cooked Vegetable Canned Vegetable _____ (particular vegetable), ex: green beans, mushrooms (raw or canned), cucumber, asparagus Dry Grass or Decayed, Dry Grass Decaying Vegetation, wet or dry Vegetation category	Burned Wire or Metal Burning Rubber Burnt Oil Smoky - wood fire smoke Smoked, cured meat Charcoal burning Heated Dust Burning category
Cherry Almond Citrus Lemon-like Watermelon Fruity category	Marine-type fish (like mackerel, cod) Decaying fish (like 2 or 3 day old fish) Shrimp Name of a type of seafood Fishy category	Fermented milk Yogurt Buttermilk Boiled milk Rotten cheese Dairy category
Roasted Potato Skin Roasted chicken Roasted meat Frying odor - corn or Bacon Roasted Nuts Roasted category	Boiled grain Toasted grain or cereal Toasted Bread Crust (possibly honey-like) Burnt grain Animal feed Grain category	Others: Shoe Polish Brown Paper Bag (cardboardy) Soap Suds odor Camphor-like(<i>Campho- phenique</i> reference) Dill weed
Geranium Violets Cedar Floral category	Bonding Glue Rubber Cement or Benzene School Paste Glue category	Beany-brewed coffee Dark-roasted coffee Burnt coffee Coffee category

APPENDIX E
GC TRACES TO SHOW REPRESENTATIVE PATTERNS OF
COMPOUNDS ELUTED FROM A TREATMENT

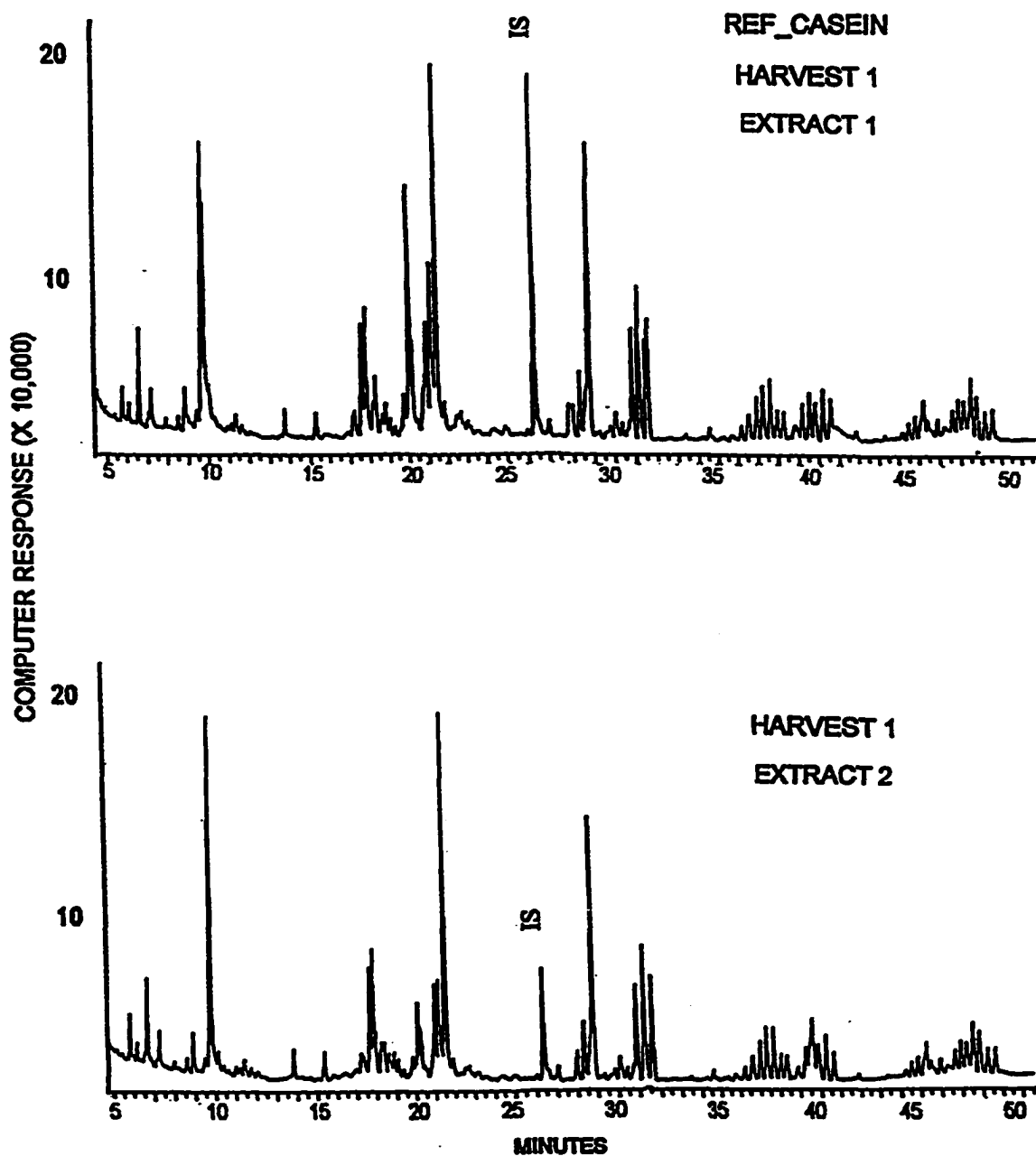


Figure 8. Gas chromatograms of volatile compounds isolated from catfish oil of REF_CASE (Harvest 1) feed treatments.

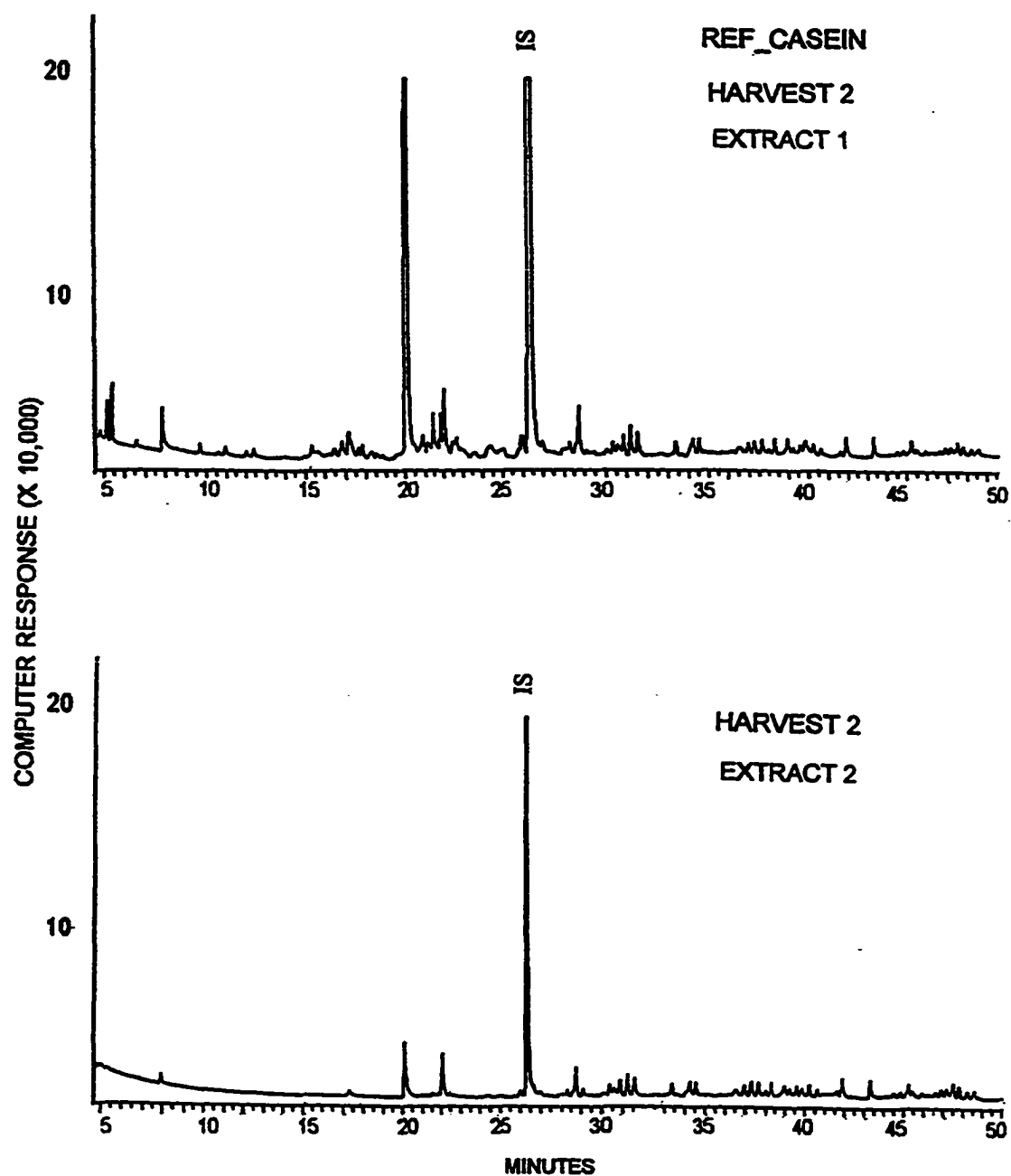


Figure 9. Gas chromatograms of volatile compounds isolated from catfish oil of REF_CASE (Harvest 2) feed treatments.

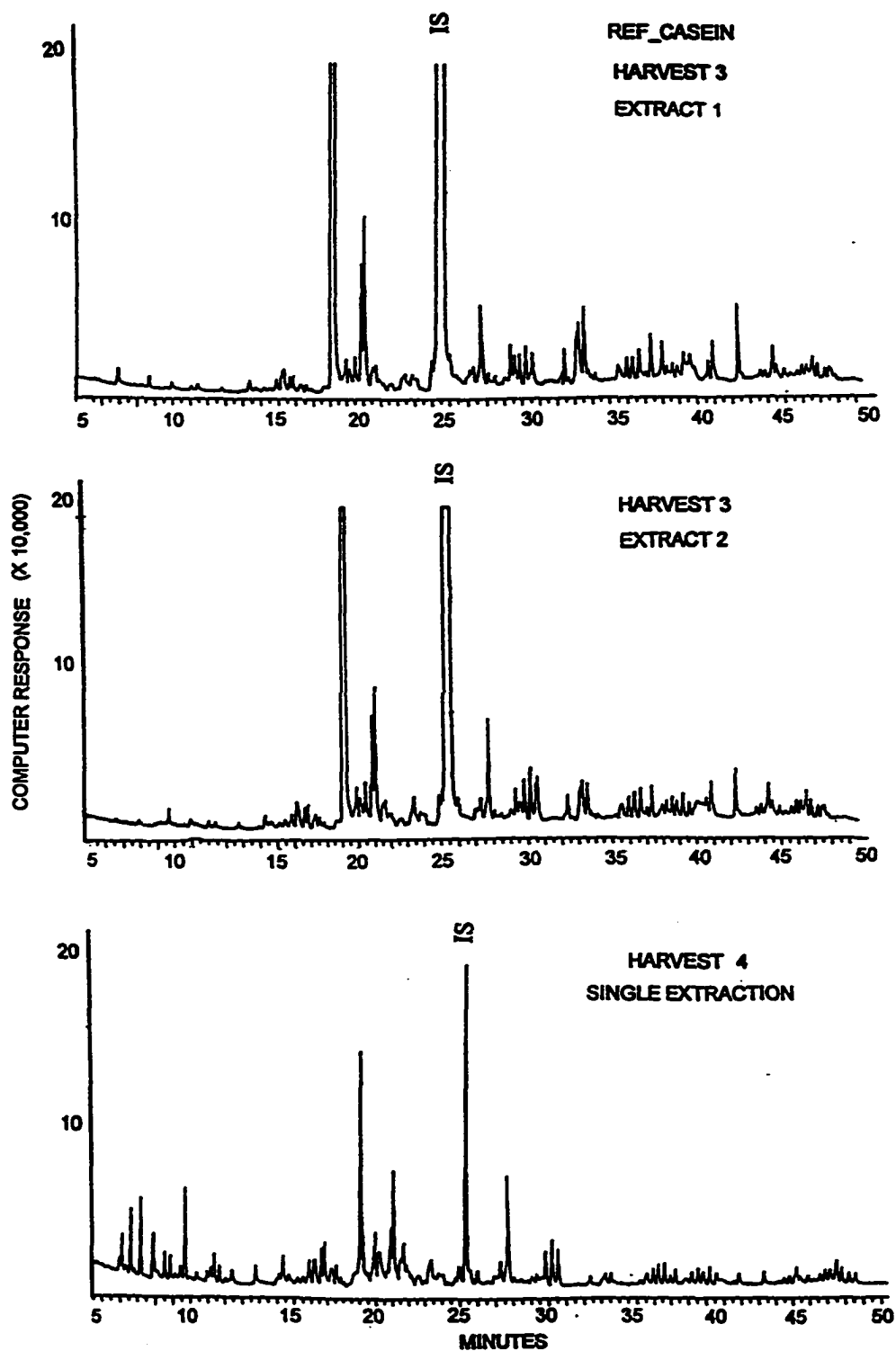


Figure 10. Gas chromatograms of volatile compounds isolated from catfish oil of REF_CASE (Harvests 3 and 4) feed treatments.

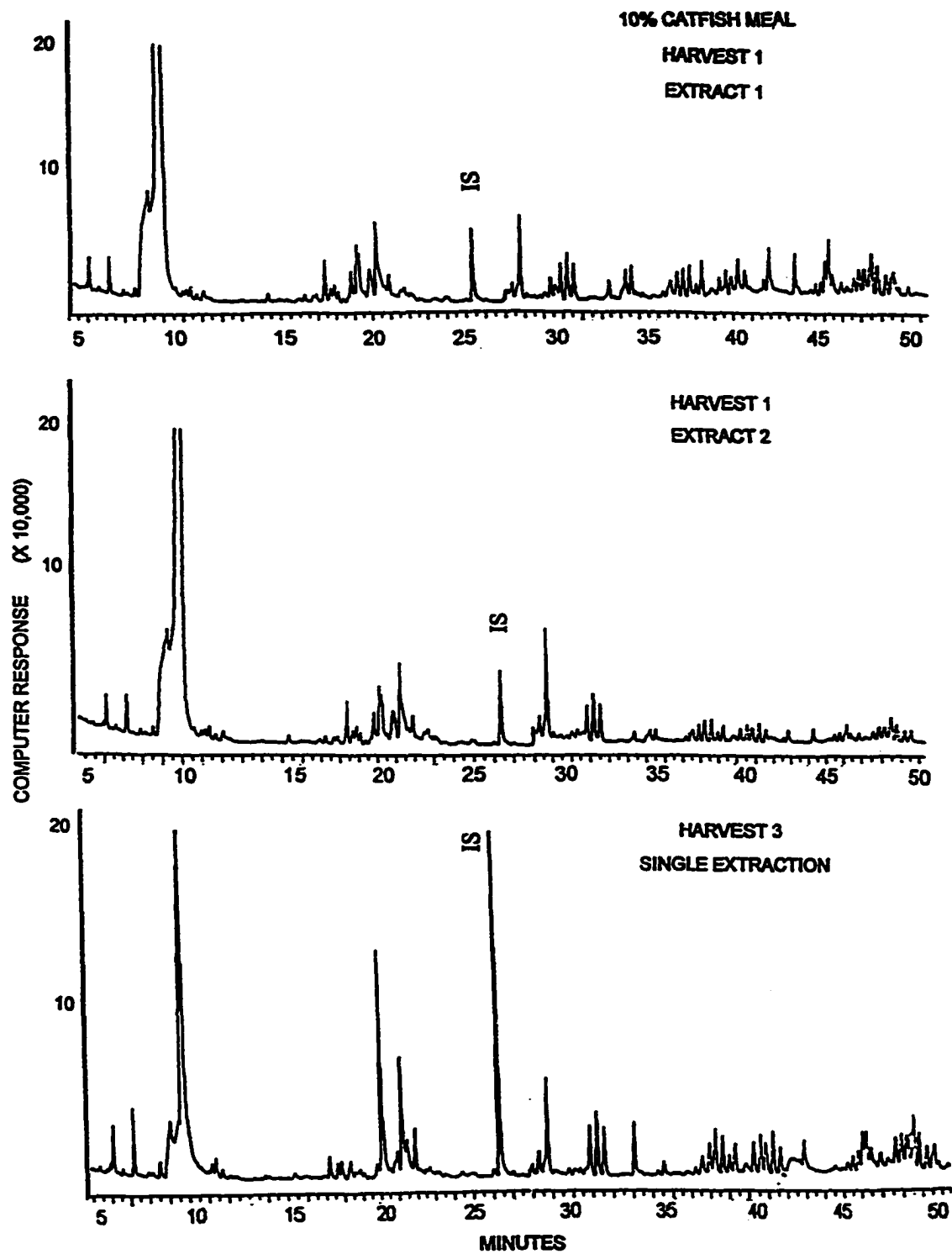


Figure 11. Gas chromatograms of volatile compounds isolated from catfish oil of CFML (Harvests 1 and 3) feed treatments.

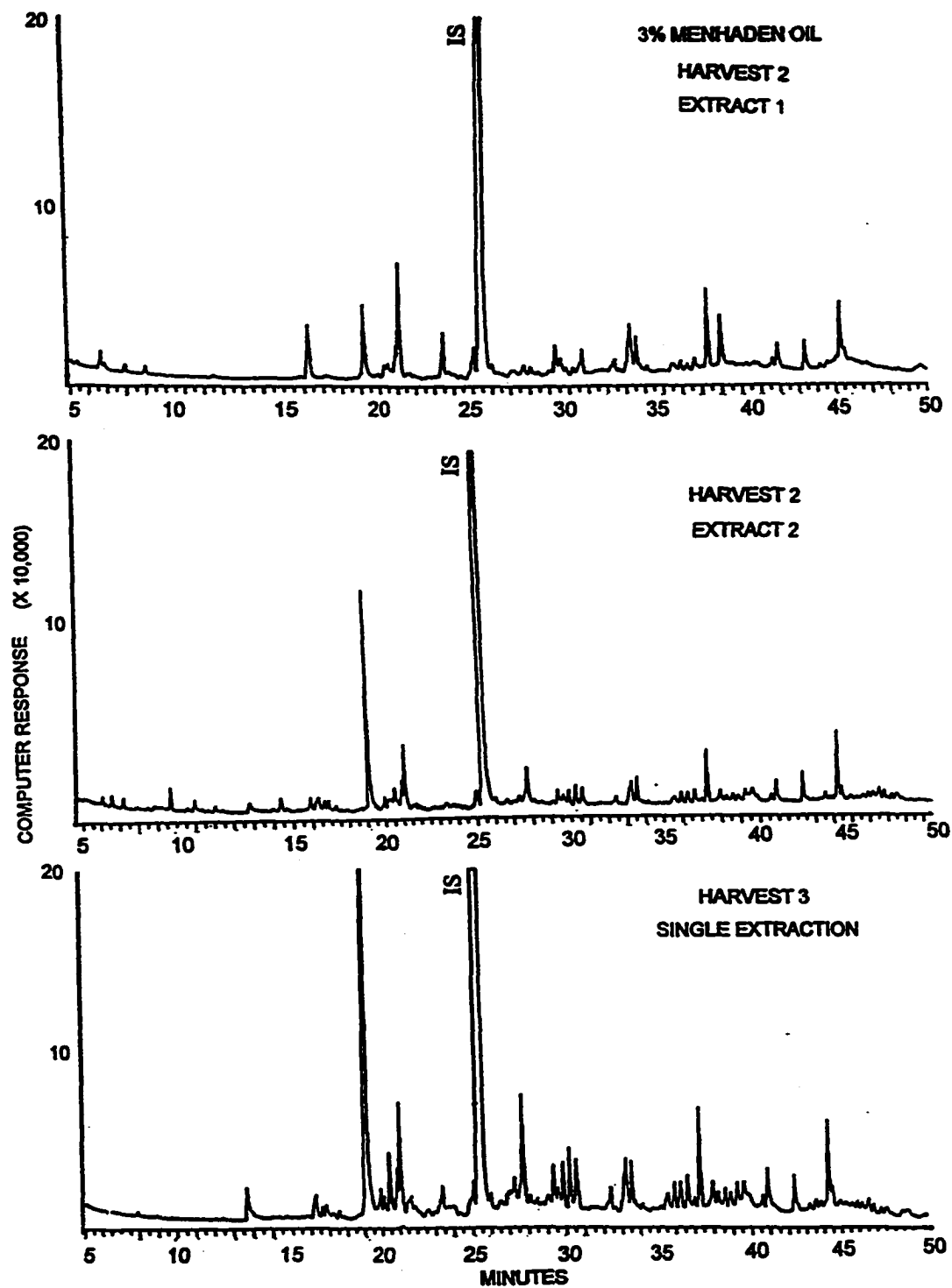


Figure 12. Gas chromatograms of volatile compounds isolated from catfish oil of MOIL (Harvests 2 and 3) feed treatments.

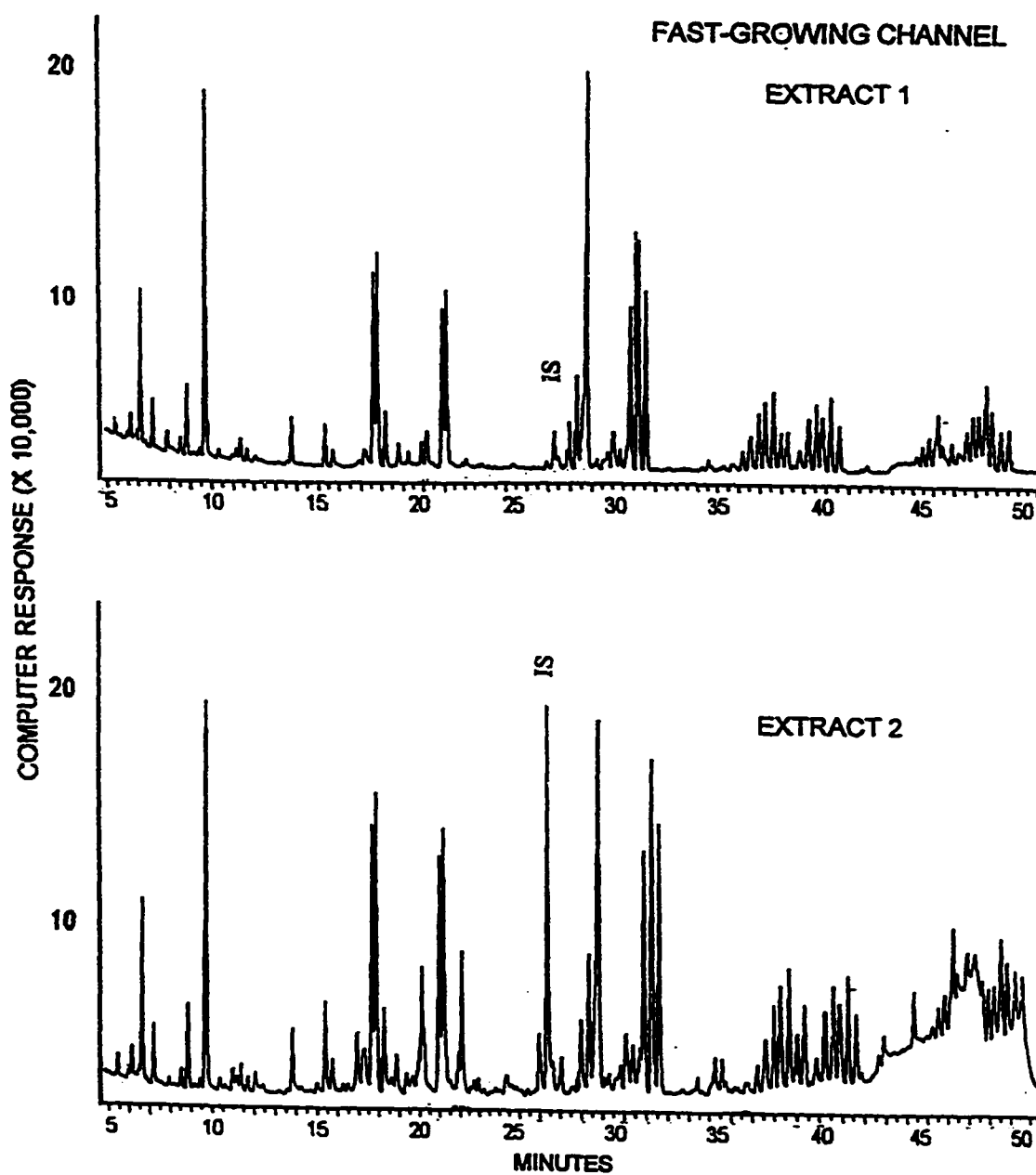


Figure 13. Gas chromatograms of volatile compounds isolated from catfish oil of FAST genetic treatment (2 extracts).

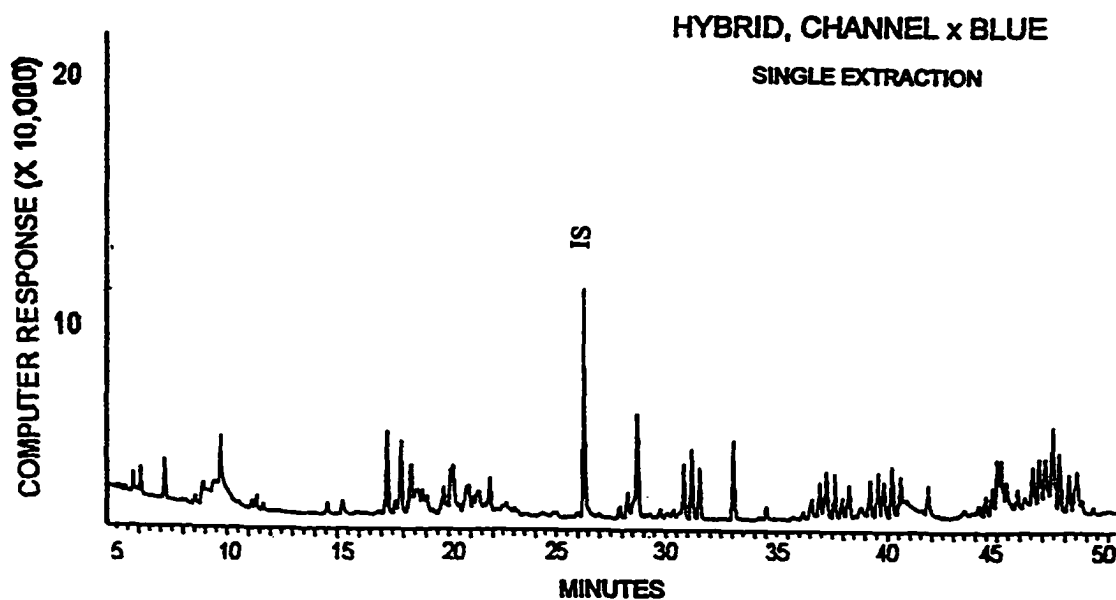


Figure 14. Gas chromatogram of volatile compounds isolated from catfish oil of HYBR genetic treatment (1 extract).

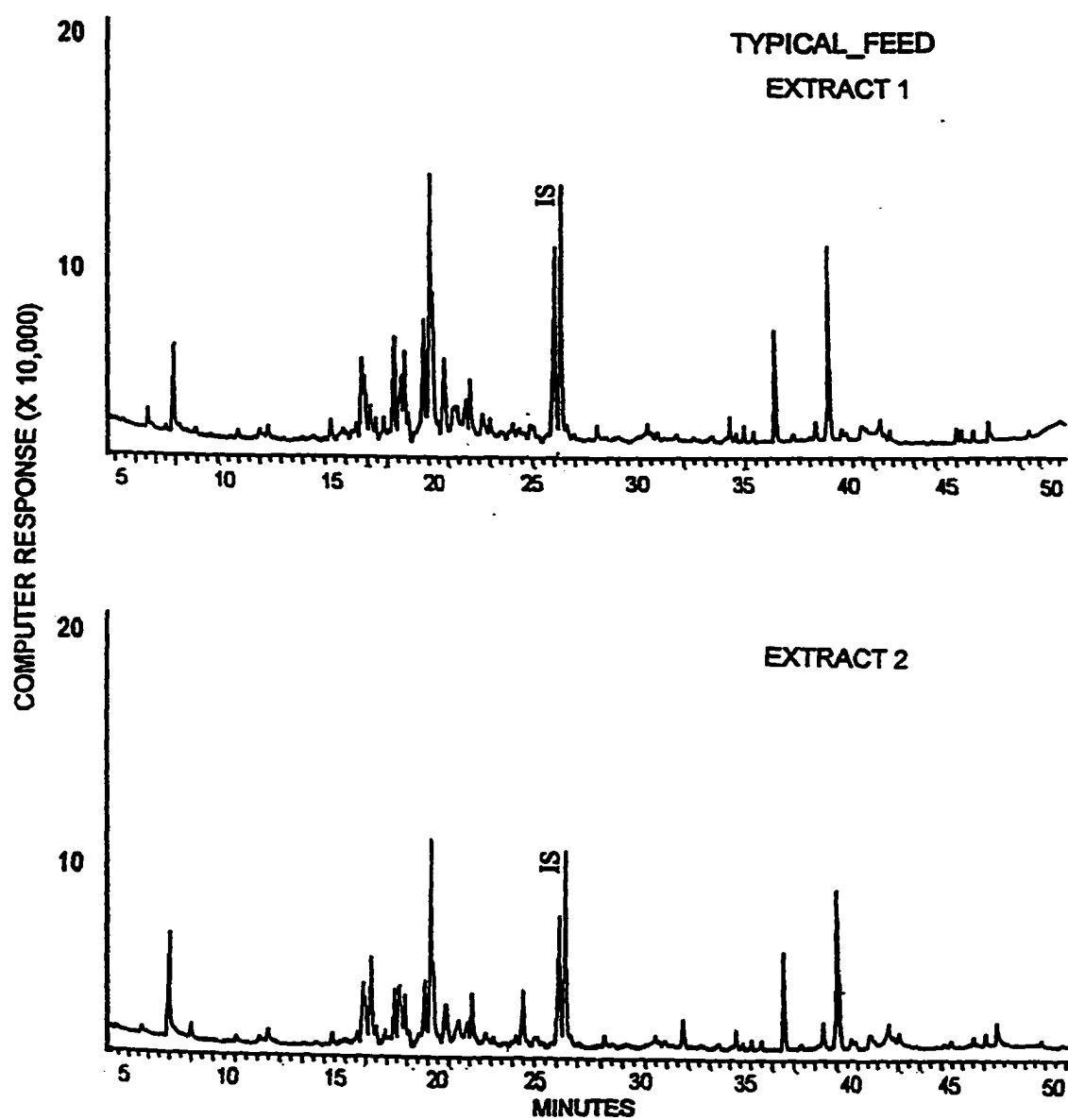


Figure 15. Gas chromatograms of volatile compounds isolated from catfish oil of TYPIC_FEED channel catfish, fresh treatment (2 extracts).

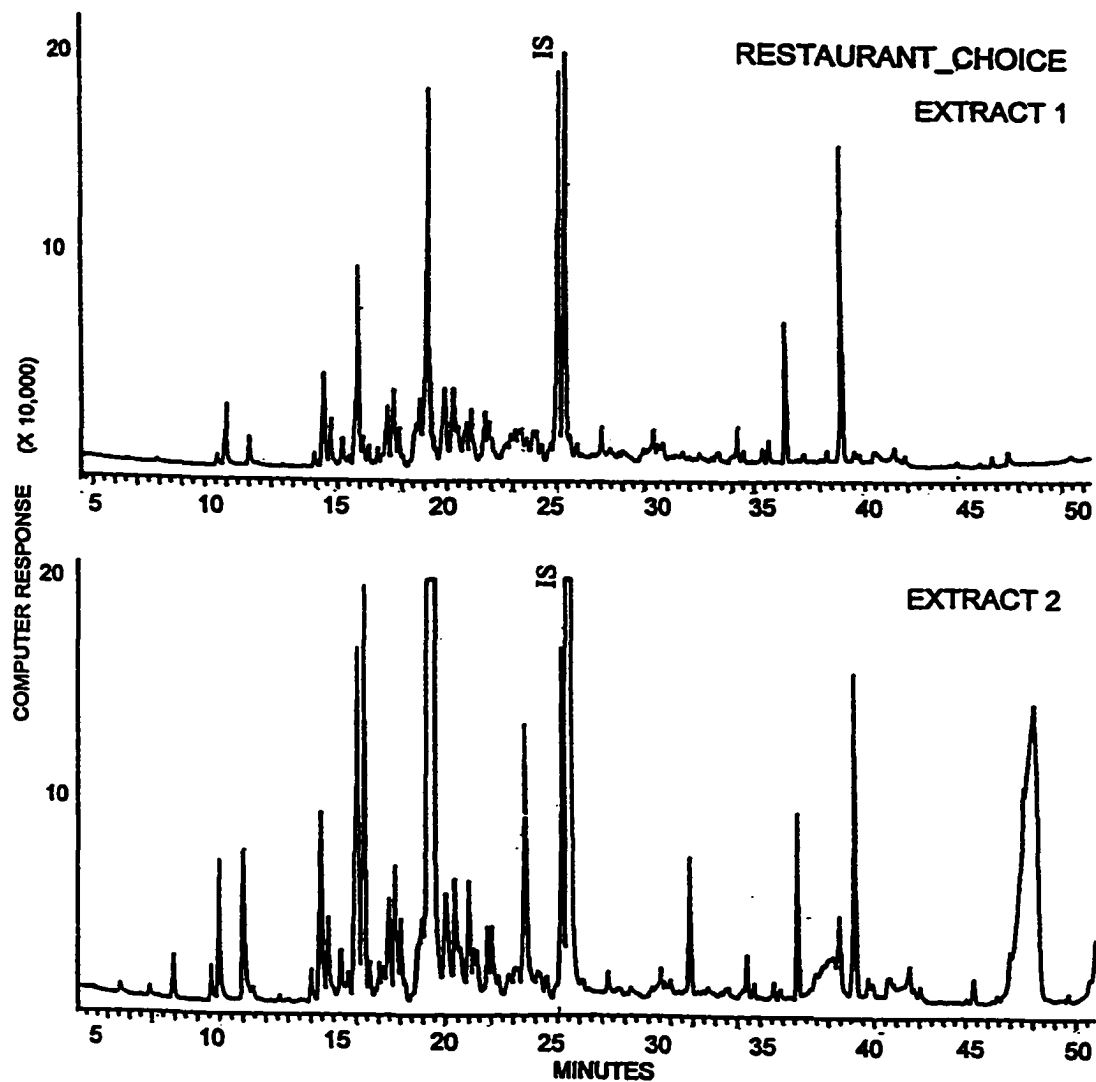


Figure 16. Gas chromatograms of volatile compounds isolated from catfish oil of REST_CHOI channel catfish, fresh treatment (2 extracts).

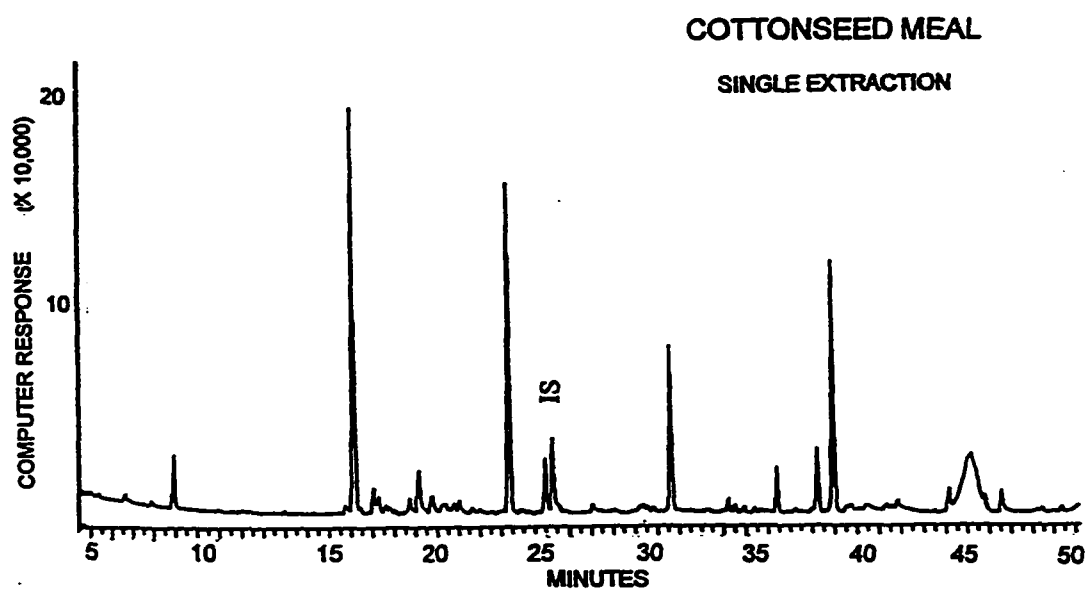


Figure 17. Gas chromatogram of volatile compounds isolated from catfish oil of COTTON channel catfish, fresh treatment (1 extract).

APPENDIX F
OCCURRENCE FREQUENCIES OF ODORS BY TREATMENT CATEGORY

Odor Label	OVER-ALL n=74	ALL FROZEN n=58	CASEIN n=25	CFML n=11	MOIL n=11	FAST n=8	HYBR n=3	ALL FRESH n=16	TYPC FEED n=8	REST CHOICE n=7	COTTN n=1
FIV	51.4	41.4	64.0	45.5	27.3	75.0	100.0	31.3	37.5	28.6	0.0
SIX	32.4	41.4	60.0	27.3	54.5	37.5	33.3	43.8	75.0	14.3	0.0
SEV	70.3	55.2	64.0	54.5	90.9	62.5	33.3	87.5	100.0	71.4	100.0
EIG	35.1	36.2	60.0	45.5	9.1	0.0	0.0	31.3	50.0	0.0	100.0
TEN	62.2	50.0	64.0	54.5	63.6	50.0	100.0	62.5	62.5	71.4	0.0
ELE	29.7	25.9	36.0	18.2	36.4	25.0	0.0	31.3	25.0	42.9	0.0
TWEL	68.9	48.3	64.0	45.5	63.6	75.0	66.7	93.8	87.5	100.0	100.0
THIRT	64.9	48.3	64.0	36.4	72.7	62.5	33.3	87.5	87.5	85.7	100.0
FIFT	67.6	48.3	56.0	54.5	72.7	75.0	33.3	93.8	100.0	85.7	100.0
SIXT	95.9	75.9	96.0	90.9	90.9	100.0	100.0	100.0	100.0	100.0	100.0
SEVT	87.8	75.9	92.0	90.9	100.0	75.0	66.7	81.3	87.5	71.4	0.0
NINT	67.6	55.2	64.0	54.5	90.9	75.0	33.3	68.8	75.0	71.4	100.0
TWEN	73.0	63.8	68.0	90.9	90.9	75.0	66.7	56.3	50.0	71.4	0.0
TTWO	74.3	63.8	68.0	100.0	81.8	62.5	100.0	62.5	62.5	57.1	100.0
TTHR	62.2	37.9	64.0	90.9	63.6	62.5	33.3	43.8	37.5	57.1	0.0
TFIV	83.8	65.5	76.0	72.7	100.0	100.0	100.0	81.3	87.5	85.7	0.0
IS		benzothiophene									
TSIX	70.3	55.2	56.0	72.7	90.9	75.0	100.0	68.8	62.5	71.4	100.0
TSEV	74.3	60.3	72.0	72.7	81.8	75.0	100.0	68.8	50.0	85.7	100.0
TNIN	56.8	53.4	68.0	63.6	63.6	37.5	33.3	43.8	37.5	42.9	100.0
THIR	89.2	74.1	96.0	81.8	90.9	62.5	100.0	93.8	87.5	100.0	100.0

VITA

Carol Ann Kelly is a native of Wilmington, Delaware, part of the urban East Coast with colonial American roots and a unique spirit. After attending the University of Delaware and The University of Texas at Austin to complete a Bachelor of Science with Honors in Nutrition and Dietetics, she served in therapeutic dietetics at a general Houston hospital.

Ms. Kelly aspired to other jobs in business, including office management and industrial customer service. While completing a Master of Science in Food Science at Louisiana State University, she also worked in hotel guest services. Subsequently, as a support food scientist at the USDA-ARS-Southern Regional Research Center in New Orleans, she received the opportunity to execute this adjunct catfish flavor research. Ms. Kelly entered the doctoral program in Food Science as a part-time student, and maintained this work-study curriculum with determination and curiosity. The program provided excellent exposure to systems in science, business and geography. During this period an invitation to serve 2 years as a practicing sensory scientist was extended by the New Zealand Dairy Research Institute. The contract period was a unique opportunity of creative work and travel. Life's surprises seasoned all these ventures along the way.

At present, Ms. Kelly has adopted a new city and culture as home. Employed by Starbucks Coffee Company on their R&D sensory team, she enjoys a lively, complex job (like their coffee), and resides in Seattle, Washington.

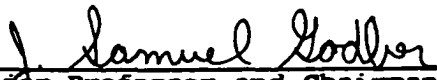
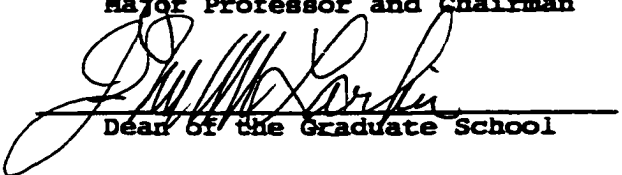
DOCTORAL EXAMINATION AND DISSERTATION REPORT

Candidate: Carol Ann Kelly


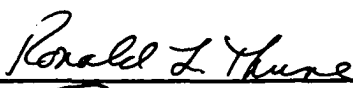
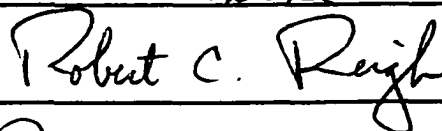
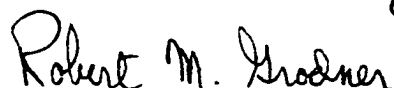
Major Field: Food Science

Title of Dissertation: Influence of Diet and Genetic Strain on Desirable
Flavors in Farm-Raised Catfish

Approved:


Major Professor and Chairman

Dean of the Graduate School

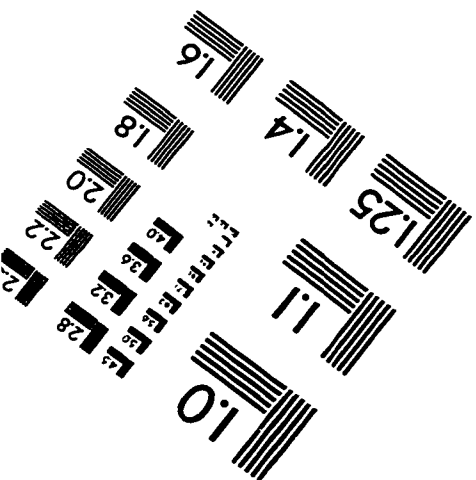
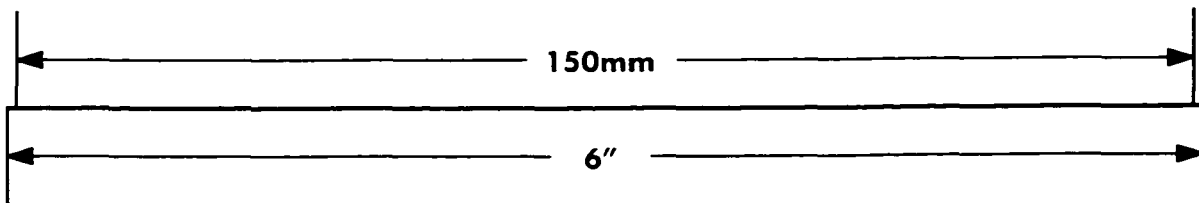
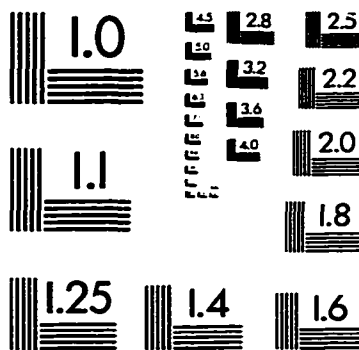
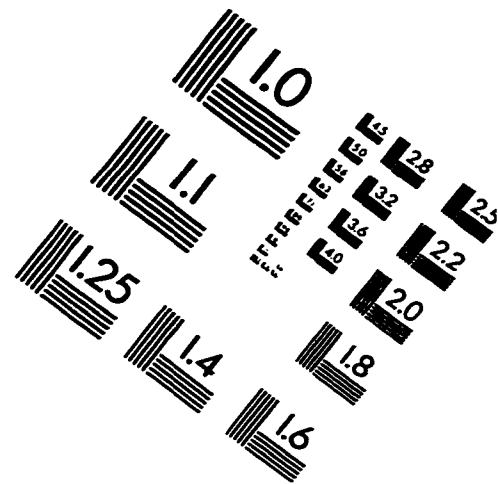
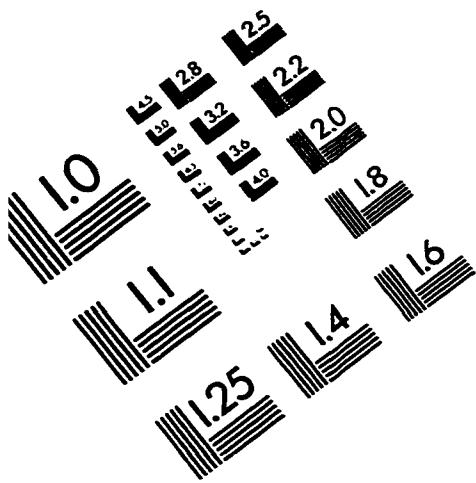
EXAMINING COMMITTEE:

Date of Examination:

March 4, 1998

IMAGE EVALUATION TEST TARGET (QA-3)



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